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Evaluation of RSDL, M291 SDK, 0.5% Bleach, 1% Soapy Water and SERPACWA:

Part 12: Challenge with EA1212 (GF, cyclosarin)

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14. ABSTRACT
An important objective of the Project Solicitation for Agent Therapeutics (Task Area 2.H) was to determine the effectiveness of current medical countermeasures against the acute toxicity of nerve agents. This project evaluated the efficacy of skin decontamination products and barrier skin creams. Doctrine described the use of Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA) as a barrier skin cream and the M291 Skin Decontamination Kit (SDK), 0.5% hypochlorite solution (household bleach diluted 1 to 10) and1% soapy water solution (liquid dish detergent) to decontaminate intact skin exposed to chemical warfare agents. Reactive Skin Decontamination Lotion (RSDL) was approved by the FDA in 2003 and subsequently selected as the Joint Services Personnel Skin Decontamination System (JSPDS) to replace the M291 SDK. This report, the twelfth in a series, directly compares the efficacy of the barrier skin cream SERPACWA and the four listed decontamination products in the haired guinea pig model following exposure to GF (EA1212).

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EXECUTIVE SUMMARY

An important objective of the Project Solicitation for Agent Therapeutics (Task Area 2.H) was to determine the effectiveness of current medical countermeasures against the acute toxicity of nerve agents. This project evaluated the efficacy of skin decontamination products and barrier skin creams. Doctrine described the use of Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA) as a barrier skin cream and the M291 Skin Decontamination Kit (SDK), 0.5% hypochlorite solution (household bleach diluted 1 to 10) and1% soapy water solution (liquid dish detergent) to decontaminate intact skin exposed to chemical warfare agents. Reactive Skin Decontamination Lotion (RSDL) was approved by the FDA in 2003 and subsequently selected as the Joint Services Personnel Skin Decontamination System (JSPDS) to replace the M291 SDK. This report, the twelfth in a series, directly compares the efficacy of the barrier skin cream SERPACWA and the four listed decontamination products in the haired guinea pig model following exposure to GF (EA1212).

In all experiments, guinea pigs were close-clipped and given anesthesia (ketamine and xylazine). SERPACWA was applied as a thin coating (0.1 mm thick), allowed to dry for 15 minutes and challenged with neat GF. After a 2-hour challenge any remaining GF was blotted off the animal, but no additional decontamination was done. In decontamination experiments, the animals were challenged with neat GF and decontaminated after a 2-minute delay for the standard procedure or at longer times for the delayed-decontamination experiments. Positive control animals were challenged with GF in the same way as the treated animals except that they received no treatment. A few of the positive control animals were challenged with 5% GF in isopropyl alcohol solution, but data from these experiments were not included in the data analysis. All animals were observed during the first 4 hours and again at 24 hours postexposure for signs of toxicity and death. The protective ratio (PR, defined as LD_{50} of the treatment group divided by the LD_{50} of the untreated positive control animals) was calculated from the derived median lethal dose-response curve parameters established for each treatment group and non-treated control animals. Significance in this report was defined as $p < 0.05$ unless otherwise stated.

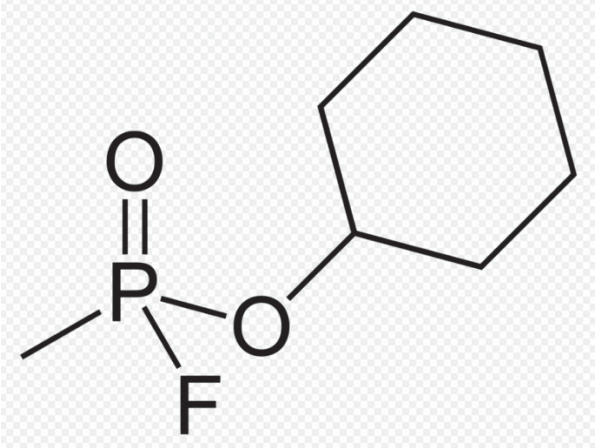
The results showed that SERPACWA provided superior protection against GF. The PR was estimated to be greater than 250. In the standard 2-minute decontamination experiments the calculated PR values (95% CI) for 0.5% bleach, M291 SDK, RSDL, and 1% soapy water were **6.82** (5.13, 9.09), **3.77** (2.64, 5.40), **25.9** (19.9, 33.6), and **6.04** (4.90, 7.44), respectively. All of the tested decontamination products provided significant protection against a GF challenge compared to untreated positive control animals. RSDL was the most effective decontamination product tested and significantly better than any of the other products. Bleach (0.5%) and soapy water (1%) provided equivalent protection and were significantly better than the M291 SDK.

In the neat GF delayed-decontamination experiments, the estimated LT_{50} (95% CI) values (the delayed-decontamination time where 50% of the animals died in the test

population following a 5 LD_{50} challenge dose of 38.8 mg/kg) for 0.5% bleach, RSDL, and 1% soapy water were **0.234** (0.065, 0.845), **34.6** (28.3, 41.7), and less than **0.5** minutes, respectively. For GF, the window for effective decontamination for 0.5% bleach and 1% soapy water was extremely short, less than 0.5 minutes; however, the window for effective decontamination using RSDL was around 35 minutes for 50% survival. A more preferable time, however, is the time in which few deaths are expected. The 10% lethality delay time (95% CI) for RSDL was **24.2** (13.9, 29.3) minutes.

INTRODUCTION

This report, the twelfth in a series, directly compares the efficacy of the four listed decontamination products and SERPACWA in the haired guinea pig model following exposure to GF [cyclosarin, EA1212, Cyclohexyl methylphoshonofluoridate, (Fluoro-methyl-phosphoryl)oxycyclohexane]. Part 1 of the series (Braue et al., 2009) provided a detailed introduction to the decontamination products, Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA), and the nerve agents, as well as to the threat nerve agents represent for warfighters and the civilian population. The first documented use of nerve agents in armed combat was during the Iran-Iraq War of 1980-1988 (Newmark, 2004). More than 100,000 US military individuals were potentially exposed to low levels of cyclosarin and sarin following the destruction of munitions at the storage facility at Khamisiyah, Iraq, in March 1991 (Smith et al., 2003).



GF (EA1212, cyclosarin)

OBJECTIVE

The first objective of this study was to determine the efficacy of four decontamination products in guinea pigs challenged with GF: 0.5% bleach, the M291 SDK, Reactive Skin Decontamination Lotion (RSDL), and 1% soapy water. The second objective was to determine how the efficacy was affected by delaying application of these decontamination products following challenge with GF. The third objective was to

determine the efficacy of the pretreatment barrier skin cream SERPACWA challenged with GF.

MATERIALS AND METHODS

A detailed description of the experimental methods used for this project may be found in the first published manuscript in the series (Braue et al., 2009). In all experiments, guinea pigs were close-clipped and given anesthesia (ketamine and xylazine). SERPACWA was applied as a thin coating (0.1 mm thick, over 7.1 cm^2), allowed to dry for 15 minutes and challenged with neat GF. After a 2-hour challenge any remaining GF was blotted off the animal, but no additional decontamination was done. In decontamination experiments, the animals were challenged with neat GF and decontaminated after a 2-minute delay for the standard procedure or at longer times for the delayed-decontamination experiments. Positive control animals were handled in the same manner as the treated animals except that they received no treatment. Four positive control animals were challenged with 5% GF in isopropyl alcohol (IPA) solution, but these animals were not included in the SAS data analysis (see discussion). All animals were observed during the first 4 hours and again at 24 hours postexposure for signs of toxicity and death. The protective ratio (PR, defined as LD_{50} of the treatment group divided by the LD_{50} of the untreated positive control animals) was calculated from the derived median lethal dose-response curve parameters established for each treatment group and non-treated control animals. Significance in this report was defined as $p < 0.05$ unless otherwise stated.

These experiments were conducted between 18 July 2006 and 14 September 2010. A 100% quality control audit was conducted by team members to verify that all data were correctly transcribed from the laboratory notebook to the Excel data base.

Agent Application

GF [cyclosarin, EA1212, Cyclohexyl methylphoshonofluoridate, (Fluoro-methyl-phosphoryl)oxycyclohexane] was obtained from the U.S. Army Edgewood Chemical Biological Center (ECBC), Aberdeen Proving Ground, MD. The experiments used lot number GF-S-6092-CTF-2 with a purity of 95.5% as determined by NMR spectroscopy.

GF was applied to the marked area on the animal (side for decontamination and back for SERPACWA) using one of various pipetting devices, depending on the volume needed. Volumes smaller than 2.0 microliters (µl) used either a Rainin micropipette (P-2, Rainin Instrument, LLC, Oakland, CA 94621) or a Hamilton microsyringe (0.5, 1, 5 µl, The Hamilton Co., Reno, NV 89502). Experiments that required volumes greater than 2.0 µl used a Rainin micropipette (P-10, P-20, P-100, P-200, P-1000) or a Drummond positive displacement microdispenser (10, 25, 50, 100, 1000 µl, The Drummond Scientific Co., Broomall, PA 19008). Either neat GF or a 5% GF IPA solution was applied, but only a total of four positive control animals received 5% GF IPA solution, and these animals were not included in the SAS data analysis.

At least once per week quality control (QC) samples of GF were prepared by pipetting 9.5 µl of neat GF into a 10 ml volumetric flask and adding chloroform to the fill line. The diluted sample of GF was analyzed by a gas chromatography (GC) system (HP model 6890) using a flame ionization detector (FID). If any sample concentration was outside three standard deviation units from the study mean the data for that day were omitted.

Data Analysis

Statistical analysis of the data from these experiments was performed using SAS software, version 6.12 (SAS Institute, Inc., Cary, NC 27513). Data sets were analyzed using specialized probit analysis programs for a stage-wise adaptive dose design (Feder, 1991) written using SAS NLIN to estimate the LD_{50} and 95% confidence interval (CI). The PROBSEP program (Braue et al., 2009, Appendix B) produced a great deal of statistical information (see APPENDIX B: SAS ANALYSIS OUTPUT FILES for complete listing), but only a small portion will be given in the tables, including LD_{10}, LD_{50}, and LD_{90} values with lower and upper 95% CIs based on Fieller's method (Finney, 1971), and the probit slope. An additional specialized program using SAS, called PRORATIO (Braue et al., 2009, Appendix C), used the output from the SAS PROBSEP program to calculate the PR of each of the treatments compared to the positive controls and to each of the other treatment groups. The PRORATIO program also estimated a confidence interval for the PR (using the delta method, Nelson, 1982), which was used to determine whether the PR was significant and therefore whether the LD_{50} values of the groups were significantly different. Statistical significance was defined as $p < 0.05$ unless otherwise stated.

The reported SAS analysis output for each treatment group used animals challenged with neat GF. The raw data tables for positive control animals included four animals challenged with 5% IPA solution of GF, but these animals were not included in the SAS analysis (see discussion).

SERPACWA data analysis presented a challenge. Twenty-two animals were initially administered doses ranging from 0.5 mg/kg to 250 mg/kg. Only one death occurred at the 25 mg/kg dose, and there were no deaths for the five animals given the 250 mg/kg dose. The death at 25 mg/kg was most likely an outlier. Doses over 250 mg/kg were difficult to apply because the volume of applied liquid was so large that some of it was likely to run off the skin site protected with SERPACWA. If GF was observed to run off the SERPACWA-protected skin, the animal was excluded from the data analysis. A combination of the EPA/OECD Acute Oral Toxicity Guideline (AOT) 425 (EPA, 2001) and a binomial probability distribution (R. Lee 2006) was used to estimate the LD_{50} for GF using the results from the 10 animals dosed at the 250 mg/kg dose (see discussion).

RESULTS

In Appendix A, Tables A1-A5 provide the raw data for the standard decontamination experiments (decontamination 2 minutes postexposure). These tables provide the 24-

hour survival data for positive control animals and animals decontaminated with 0.5% bleach, M291 SDK, RSDL, and 1% soapy water. Five percent GF IPA solution was used for only the first four positive control animals in stage 1 of dosing. All the other animals were challenged with neat GF.

In Appendix A, Table A7 provides the raw data for the SERPACWA experiments. This table provides the 24-hour survival data for animals pretreated with SERPACWA. Neat GF was used for all SERPACWA animals.

In Appendix A, Tables A7-A9 provide the survival raw data for the delayed-decontamination experiments. In delayed-decontamination experiments, the decontamination process was delayed from 0.5 to 90 minutes postexposure. All these animals were challenged with 38.8 mg/kg BW (body weight), representing a 5 LD_{50} dosage.

Appendix B provides the SAS output files giving complete details of the data analysis. A fixed limit statistical test was used for the SERPACWA animals so there was no SAS output.

Figure 1 is a graph of the LD_{50} values for the four decontamination products and positive control animals in the guinea pig model. The error bars represent the 95% CI. The number of animals used per treatment group was between 21 and 35. All animals were challenged with neat GF. The LD_{50} values (95% CI) for positive control, 0.5% bleach, M291 SDK, RSDL, and 1% soapy water were **7.71** (2.51, 10.7), **52.6** (43.4, 9.26), **29.1** (20.1, 42.3), **200** (153, 252), and **46.6** (23.0, 52.0) mg/kg BW, respectively.

Figure 2 contains dose-response curves for decontamination products in the guinea pig model. The LD_{50} values (mg/kg BW) are given in the text blocks. The dose-response curve slopes (95% CI) for positive control, 0.5% bleach, M291 SDK, RSDL, and 1% soapy water were **6.94** (-0.224, 14.1), **7.45** (1.88, 13.0), **4.43** (1.84, 7.02), **6.96** (2.10, 11.8), and **17.6** (-0.004, 35.2), respectively.

Figure 3 is a graph of the PR values with respect to the positive controls. The PR values (95% CI) for 0.5% bleach, M291 SDK, RSDL, and 1% soapy water were **6.82** (5.13, 9.09), **3.77** (2.64, 5.40), **25.9** (19.9, 33.6), and **6.04** (4.90, 7.44), respectively. PR values with the same letter were not significantly different.

In the SERPACWA efficacy experiments all animals were challenged with neat GF. The LD_{50} value (95% CI) for control animals (same animals as the standard 2-minute decontamination experiments) was **0.771** (2.51, 10.7). The LD_{50} value for SERPACWA animals was estimated to be > 250 mg/kg BW by using a combination of a fixed and sequential limit test and observing 1 death in 10 animals all challenged at 250 mg/kg BW. SERPACWA provided excellent protection.

Figure 4 is a graph of percent lethality when 0.5% bleach, RSDL, and 1% soapy water decontamination was delayed following challenge by 38.8 mg/kg (5 LD_{50}s) of neat

GF. LT_{50} (50% lethality time with 95% CI) values for bleach and RSDL were **0.234*** (0.065, 0.845) and **34.6** (28.3, 41.7) minutes, respectively. *The LT_{50} for bleach was estimated using the less conservative delta method instead of the standard, very conservative Fieller's method. The LT_{50} value for 1% soapy water was estimated to be < 0.5 minutes by using a sequential limit test and observing 9 out of 10 deaths at a delay time of 0.5 minutes. The dose-response curve slopes (95% CI) for 0.5% bleach and RSDL were **2.04** (-0.270, 4.34) and **8.28** (3.07, 13.5), respectively.

Tables 1-3 provide a summary of the SAS probit analysis for all of the experiments. It consists of, for each treatment, the number of animals, the LD_{10}, LD_{50} and LD_{90} (LT_{10}, LT_{50} and LT_{90} for delayed-decontamination experiments), the lower and upper 95% CI, the dose-response curve slope, and the PR. The delta method was used to calculate confidence intervals for PRs and to determine which were significantly different.

AGENT	No. of G.P.	Treatment	LD_{50} mg/kg (95% C.I.)	Slope (95% C.I.)	PR (95% C.I.)	Sig p < 0.05		LD_{10} mg/kg (95% C.I.)		LD_{90} mg/kg (95% C.I.)
GF Neat	28	Control	**7.71** (2.51 - 10.7)	**6.94** (-0.224 – 14.1)	**1.00**	**a**		**5.04** (0.0003 - 6.55)		**11.8** (9.36 - 40112)
GF Neat	28	Bleach	**52.6** (43.4 - 86.4)	**7.45** (1.88 - 13.0)	**6.82** (5.13 – 9.09)	**b, e**		**35.4** (18.4 - 43.0)		**78.2** (59.5 - 298)
GF Neat	35	M291	**29.1** (20.1 - 42.3)	**4.43** (1.84 - 7.02)	**3.77** (2.64 - 5.40)	**c**		**15.0** (5.82 - 21.3)		**56.6** (39.7 - 147)
GF Neat	32	RSDL	**200** (153 - 252)	**6.96** (2.10 - 11.8)	**25.9** (19.9 - 33.6)	**d**		**131** (51.8 - 165)		**305** (244 - 708)
GF Neat	21	Soap	**46.6** (23.0 - 52.0)	**17.6** (-0.004 - 35.2)	**6.04** (4.90 - 7.44)	**e, b**		**39.4** (2.30 - 44.6)		**55.1** (50.0 - 278)

Table 1. Data summary of efficacy experiments for decontamination products with animals challenged with GF. Neat GF was used for all animals. Data includes standard 2-minute decontamination experiments from 18 July 2006 to 29 August 2006 (Tables A1 – A5).

AGENT	No. of G.P.	Treatment	LT_{50} min (95% C.I.)	Slope (95% C.I.)	Sig p < 0.05		LT_{10} min (95% C.I.)		LT_{90} min (95% C.I.)
GF Neat	40	Bleach delayed	**0.234*** (0.0651 - 0.845)	**2.04** (-0.270 - 4.35)	**a**		**0.0552*** (0.00375 - 0.814)		**0.996*** (0.404 – 2.45)
GF Neat	37	RSDL delayed	**34.6** (28.3 - 41.7)	**8.28** (3.07 - 13.5)	**b**		**24.2** (13.9 - 29.3)		**49.4** (41.2 - 82.3)
GF Neat	27	Soap delayed	**<0.5**	**NA**	**NA**		**NA**		**NA**

Table 2. Data summary of efficacy experiments for delayed decontamination with animals challenged with 38.8 mg/kg BW neat GF (5 LD_{50}s). * The LT_{50} for bleach was estimated using the less conservative delta method instead of the standard, very conservative Fieller's method. The LT_{50} value for 1% soapy water was estimated to be < 0.5 minutes by using a sequential limit test and observing 9 out of 10 deaths at a delay time of 0.5 minutes. Data includes delayed-decontamination experiments from 28 July 2010 to 14 September 2010 (Tables A7 – A9).

AGENT	No. of GP	Treatment	LD_{50} mg/kg (95% C.I.)	Slope	PR (95% C.I.)	Sig p < 0.05		LD_{10} mg/kg (95% C.I.)		LD_{90} mg/kg (95% C.I.)
GF Neat	28	Control	**7.71** (2.51 - 10.7)	**6.94** (-0.224 – 14.1)	**1.00**	**a**		**5.04** (0.0003 - 6.55)		**11.8** (9.36 - 40112)
GF Neat	27	SERPACWA	**>250**	**NA**	**>32.2**	b		**NA**		**NA**

Table 3. Data summary of efficacy experiments for SERPACWA with animals challenged with neat GF. The LD_{50} value for SERPACWA animals was estimated to be > 250 mg/kg BW by using a combination of a fixed and sequential limit test and observing 1 death in 10 animals all challenged at 250 mg/kg BW. Data includes SERPACWA experiments from 18 July 2006 to 23 August 2006 (Tables A1 and A6).

Notes for Tables 1-3:

- LD_{10}, LD_{50}, and LD_{90} = the dosage (mg/kg BW) required to kill 10, 50, and 90%, respectively, of the test population by 24 hours postexposure (95% CI using Fieller's method).
- LT_{10}, LT_{50}, and LT_{90} = the delayed-decontamination time at which 10, 50, and 90% of the animals in the test population die by 24 hours postexposure (95% CI using Fieller's method).
- PR = Protective ratio (LD_{50} of treatment/LD_{50} of control) with 95% CI using the delta method.
- Sig = Protective ratios with same letter were not significantly different ($p < 0.05$; using the delta method).
- Slope = The slope of the dose-response curve with 95% CI using Fieller's method.
- NA = Not available

DISCUSSION

Decontamination Efficacy Experiments

The four decontamination products evaluated can be divided into two categories based on cost and use. The M291 SDK and RSDL are relatively expensive (the RSDL packet costs about $20 and the M291 SDK cost about $3.35) and designed to be carried by individuals for immediate lifesaving spot decontamination on small intact skin areas following exposure. The U.S. military began to phase out the use of the M291 SDK in 2008, and it is no longer commercially available. On the other hand, 0.5% bleach and 1% soapy water are relatively inexpensive and generally used for whole body decontamination of an exposed person prior to moving the individual from a dirty zone into a clean zone. All four of these products were described in U.S. doctrine for CWA decontamination of intact skin; however, a comprehensive evaluation comparing the efficacy of these products was never accomplished prior to this study. This technical report is the twelfth in a series to provide a comprehensive comparison of the efficacy of these decontamination products and SERPACWA against many of the traditional agents and other toxic compounds.

The real world threat scenario is for exposure to neat agent not agent in solution. The toxicity of many nerve agents was so great, however, that the dosage volumes required to cover the full range of the dose-response curve for untreated animals were too small to be pipetted reliably. Initially, the decision was made to minimize the pipetting uncertainties in precision and accuracy by using neat agent only whenever the challenge dose required a volume greater than or equal to 0.5 µl.

During the evaluation of toxic compounds, we discovered that the efficacy of a decontamination product can be greatly affected by whether the agent is neat or in solution. We observed that the decontamination products were significantly less effective for animals challenged with agent in solution than when the agent was neat. We theorized that this observation resulted from the solvent increasing the systemic uptake of agent by changing the barrier function of the skin and by spreading the agent over a larger skin area, thus making the decontamination process less effective. These observations caused us to re-think our decision to use agent in solution when evaluating cutaneous treatments. Following this analysis, the decision was made in November 2005 to use only a neat agent challenge for evaluating skin treatments in all future experiments. We recognized that trying to pipette very small volumes (0.05 to 0.5 µl) could not be accomplished with a high degree of accuracy or precision. We decided it was better to use neat agent and accept this uncertainty than to generate unreliable data from using agent in solution. The uncertainty in delivering these very small volumes was mitigated to some degree by using more animals for each experiment. For positive control animals, which received no treatments, agent in solution was still used if the required volume was < 0.5 µl. The rationale for this decision was that the agent had 24 hours to reach the systemic circulation; thus a modified penetration rate should not significantly affect the observed 24-hour lethality.

The first day of the GF study was a dose-ranging experiment that used a 5% solution of GF in isopropyl alcohol (IPA) for positive control animals and neat GF for all treatment animals. We observed that GF in untreated animals was much less toxic than many of the other nerve agents, so for the remainder of the study all animals, positive controls and treatment groups, were challenged with neat GF. For consistency, the four initial animals that were challenged with a 5% solution of GF in IPA were omitted from the SAS statistical analysis.

In the standard 2-minute decontamination experiments the calculated PR values (95% CI) for 0.5% bleach, M291 SDK, RSDL, and 1% soapy water were **6.82** (5.13, 9.09), **3.77** (2.64, 5.40), **25.9** (19.9, 33.6), and **6.04** (4.90, 7.44), respectively. All of the tested decontamination products provided significant protection against a GF challenge compared to untreated positive control animals. RSDL was the most effective decontamination product tested and significantly better than any of the other products. Bleach (0.5%) and soapy water (5%) provided equivalent protection and were significantly better than the M291 SDK.

The LD_{50} value is traditionally used to compare the toxicity of chemicals; however, the dose-response curve slope is also an important parameter to indicate how quickly the percent lethality changes with applied dose. If the dose-response curve slope is flat, the percent of lethality changes very slowly with changes in dose, and a significant percentage of deaths is observed at doses far removed from the LD_{50} dose. The PROBSEP program run in SAS to analyze this data set not only provided the LD_{50} values but also gave doses for the complete range of lethality percentiles including 1, 10, 16, 30, 50, 70, 84, 90, and 99. The doses for this entire range are listed in the SAS output files recorded in Appendix B. Table 1 provides the slope, LD_{10} LD_{50}, and LD_{90} values along with the 95% CI values so that the reader can fully understand the toxicity of GF and the effectiveness of the products tested.

In a real-life scenario, warfighters or civilians may not realize that they have been contaminated with a toxic agent. Thus, they may not start the decontamination process until well after the recommended time of 1 or 2 minutes postexposure. The conventional wisdom for many years was that decontamination would only be effective if performed in the first few minutes after exposure. When this study started in 2005, no comprehensive evaluations were available on the effectiveness of decontamination products beyond the standard 2-minute delay time. A limited study (Hamilton et al., 2004) using only 3 animals per treatment group evaluated VX decontamination with RSDL in swine (Yorkshire-Landrace cross, 20 kg). In the Hamilton et al. study, RSDL was found to be significantly effective 15 minutes postexposure for neat VX challenge to the ear but not significantly effective 30 or 60 minutes postexposure for neat VX challenge to the epigastrium (belly). Recognizing the need for a comprehensive study, the scope our current study was expanded to include delayed-decontamination studies.

A fixed neat GF challenge dose of 38.8 mg/kg BW (5 LD_{50}) was used for all GF delayed-decontamination studies. This value was based on the results for the positive control animals. We used a 5 LD_{50} challenge because historically a 5 LD_{50} dose was the

suggested minimum target for medical countermeasure therapeutics selected for fielding. The lethality delay time-response curves were generated using the stage-wise adaptive dose design similar to the LD_{50} dose-response studies using the delay time in place of the mg/kg dose. The standard SAS probit analysis program was used to find the lethality percentiles associated with a given decontamination delay time. The LT_{10}, LT_{50}, and LT_{90} values were defined as the delayed-decontamination times at which 10, 50, and 90% of the animals in the test population died following a 38.8 mg/kg (5 LD_{50}) neat GF challenge. A PR of 5, which is directly related to protection from a 5 LD_{50} challenge, was the decision criteria for choosing the decontamination products for the delayed-decontamination experiments. Any decontamination products with a PR > 5 would be evaluated for delayed decontamination. For the GF experiments RSDL, 0.5% bleach, and 1% soapy water met this requirement. The M291 SDK with a PR of only 3.77 was omitted from the delayed-decontamination experiments.

In the neat GF delayed-decontamination experiments, the estimated LT_{50} (50% lethality time with 95% CI) values for bleach and RSDL were **0.234** (0.065, 0.845) and **34.6** (28.3, 41.7) minutes, respectively. The LT_{50} for bleach was estimated using the less conservative delta method instead of the standard, very conservative Fieller's method. There were insufficient data for the Fieller's method to produce meaningful output. The less rigorous delta method was able to use the experimental results to estimate the dose-response curve with 95% CI values. Additional animals were not used because it was unlikely that useful data would be generated. This series of experiments suggests that the window (less than 30 seconds) for effective decontamination using 0.5% bleach is very short. It should be noted that the very short window for effective decontamination using bleach was not consistent with the data generated four years earlier in the standard 2-minute decontamination experiments. The PR (95% CI) for 0.5% bleach in the standard 2-minute delay experiments was 6.82 (5.13, 9.09), suggesting that decontamination at 2-minutes postexposure would protect at least 50% of the animals following a 5 LD_{50} challenge. A possible explanation is the 4-year time difference and large variation in the observed data for the delayed-decontamination experiments.

The LT_{50} value for 1% soapy water was estimated to be < 0.5 minutes by using a sequential limit test and the Binomial Exact Probability Table and observing 9 out of 10 deaths at a delay time of 0.5 minutes. For more details about the sequential limit test and the Binomial Exact Probability Table see the discussion about SERPACWA.

For GF, the window for effective decontamination for 0.5% bleach and 1% soapy water was less than 0.5 minutes; however, the window for effective decontamination using RSDL was around 35 minutes for 50% survival. A more preferable time, however, is the time in which few deaths are expected. The 10% lethality delay time (95% CI) for RSDL was **24.2** (13.9, 29.3) minutes.

Recent studies using Raman confocal microspectroscopy (Braue et al., 2015) demonstrated that RSDL has the ability to remove VX from a VX depot in the top layers of the stratum corneum. It is likely that GF also forms a GF depot in the stratum

corneum following percutaneous exposure. The effectiveness of RSDL against GF may partially be explained by the ability of RSDL to remove the GF depot. This speculation could be confirmed by experiments if funding were available.

SERPACWA Efficacy Experiments

SERPACWA provided good protection against GF. The LD_{50} was estimated to be at least 250 with a PR estimated to be greater than 32. SERPACWA data analysis, however, presented a challenge. Twenty-two animals were initially dosed with challenges ranging from 0.5 mg/kg to 250 mg/kg. Only one death occurred at the 25 mg/kg dose, and there were no deaths for the five animals given the 250 mg/kg dose. The death at 25 mg/kg was most likely an outlier. Dosing volumes higher than 250mg/kg were likely to likely to run off the skin site protected with SERPACWA. Therefore, the AOT 425 procedures were used to estimate the LD_{50} for GF using the results from the 10 animals dosed with the 250 mg/kg dose.

The AOT 425 is used by the EPA to provide a gross classification of a chemical's toxicity. It consists of two tests, a limit test and a main test, which are sequential tests, i.e., dosing one animal at a time. The EPA uses these tests with maximum limit doses of either 2000 or 5000 mg/kg and a maximum of five animals. Based on the number of deaths among the five animals, the test determines if the LD_{50} is above or below the limit dose.

The procedures for the limit test are as follows: dose one animal at the limit dose. If the animal dies, then switch to the main test. If the animal lives, dose four additional animals sequentially at the same dose for a total of five animals at the same dose. If during the sequential dosing, three animals have died, then switch to the main test. If three or more of the five animals dosed survive, then the LD_{50} is greater than the limit dose used. If three or more animals die, then the LD_{50} is less than the limit dose used, and an estimate of the LD_{50} is made using the main test.

In this study, the first five animals that received the 250 mg/kg dose were dosed in a sequential manner along with the other animals that were dosed at lower doses. The last five animals dosed at 250 mg/kg were all dosed at once. Of those last five animals, only one died. By using the limit test approach from the AOT 425 with 250 mg/kg as the limit dose for each set of five animals, we can conclude that the LD_{50} is greater than 250 mg/kg.

Another approach is to use a binomial probability distribution (R. Lee, 2006) on both groups of animals combined, n=10. If the LD_{50} is 250 mg/kg then the death/survival rate would be 50%. We can determine the cumulative probability of observing one or fewer deaths out of ten animals using Table 4 below. We observe that the exact probabilities for 0 deaths out of ten animals is 0.1% and for 1 death out of ten animals is 0.98%. To obtain the cumulative probability, we add these two percentages together. Therefore, the cumulative probability of the 250 mg/kg dose being the LD_{50} (i.e. having a 50% death/survival rate) is 1.08%. Since this is less than a 5% error rate, we reject the assumption that 250 mg/kg is the dose at which we would see 50% of the animals die (LD_{50}) and conclude that the LD_{50} is greater than 250 mg/kg.

Total Exposed	Deaths	Probability (%)	Deaths	Probability (%)
5	0	3.13	1	15.6
6	0	1.56	1	9.4
7	0	0.78	1	5.4
8	0	0.39	1	3.1
9	0	0.20	1	1.76
10	0	0.10	1	0.98

Table 4. Binomial Probability Distribution of Observed Death

In 2009, the Department of the Army decided that there was no longer a requirement for SERPACWA, since the new JSLIST protective suit was reported to solve the problem of leaking at the junction points (neck, wrist, waist, and ankle). Thus, SERPACWA was removed from the Army's stockpile and destroyed. Since that time, new evidence has suggested that a barrier cream like SERPACWA may still be needed.

CONCLUSIONS

- RSDL provided good protection against GF with a protective ratio of **25.9** and was significantly better than the other products tested.

- The relative order of decontamination efficacy against GF was RSDL > 0.5% bleach and 1% soapy water > M291 SDK.

- The window for effective decontamination following percutaneous exposure from GF was about 35 minutes for RSDL but less than 30 seconds for 0.5% bleach and 1% soapy water.

- Skin Exposure Reduction Paste Against Chemical Warfare Agents (SERPACWA) provides superior protection against GF with a protective ratio of greater than 250.

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FIGURES

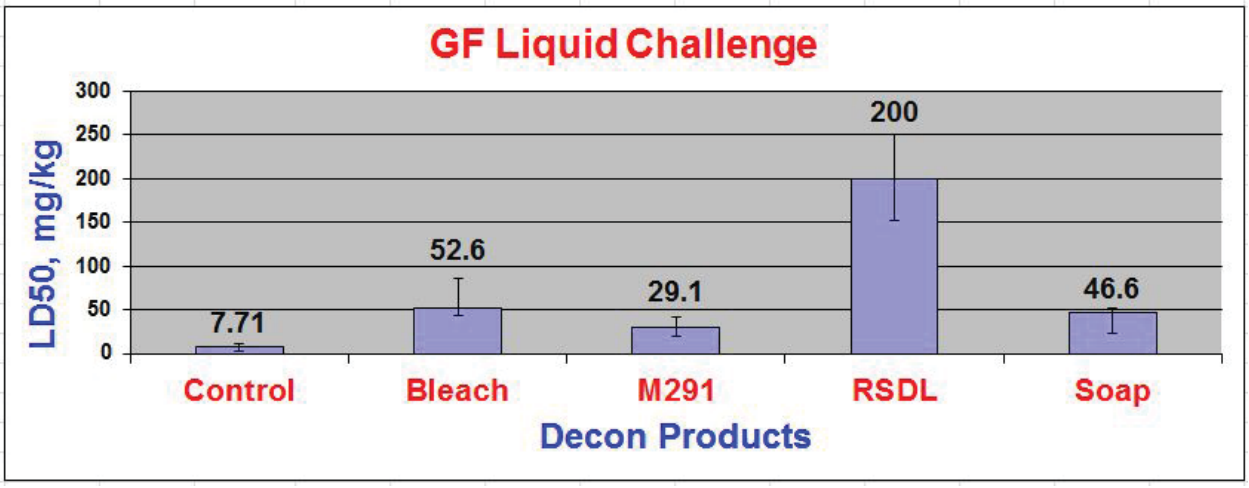


Figure 1. Graph of LD_{50} values for decontamination products in guinea pig model. Error bars = 95% CI. The numbers of animals used per treatment group were 21 - 35. Animals were challenged with neat GF. The LD_{50} values (95% CI) for positive control, 0.5% bleach, M291 SDK, RSDL, and 1% soapy water were **7.71** (2.51, 10.7), **52.6** (43.4, 9.26), **29.1** (20.1, 42.3), **200** (153, 252), and **46.6** (23.0, 52.0) mg/kg BW, respectively.

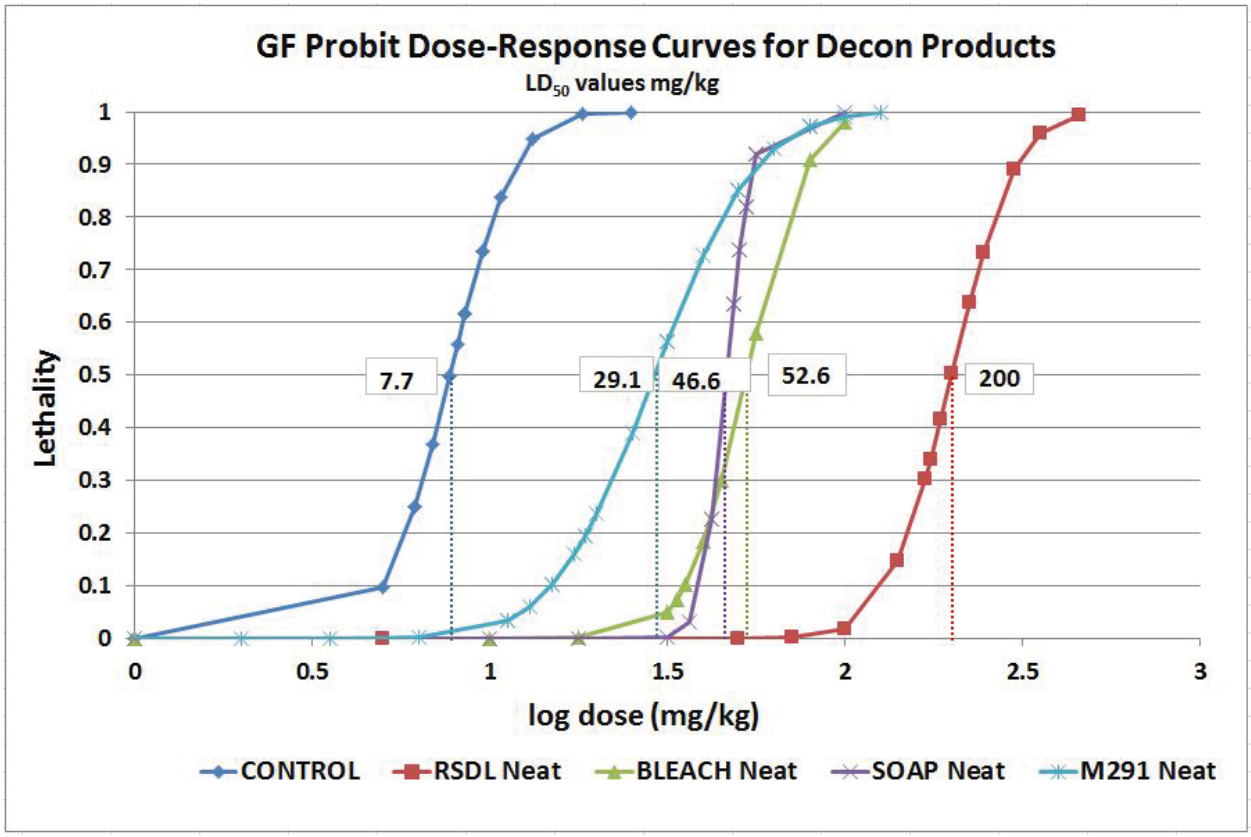


Figure 2. Dose-response curves for decontamination products in guinea pig model. Neat GF was used for all animals. LD_{50} values (mg/kg BW) are given in text blocks. Dose-response curve slopes (95% CI) for positive controls, 0.5% bleach, M291 SDK, RSDL, and 1% soapy water were **6.94** (-0.224, 14.1), **7.45** (1.88, 13.0), **4.43** (1.84, 7.02), **6.96** (2.10, 11.8), and **17.6** (-0.0037, 35.2), respectively.

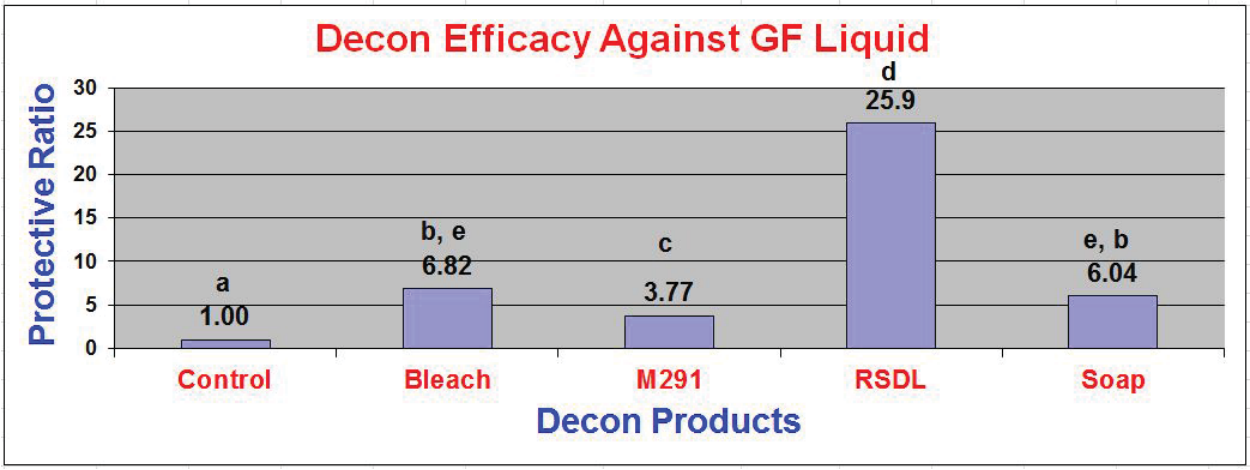


Figure 3. Graph of protective ratio (PR) values for decontamination products in guinea pig model. Animals were challenged with neat GF. The PR values (95% CI) for positive control, 0.5% bleach, M291 SDK, RSDL, and 1% soapy water were **1.00**, **6.82** (5.13, 9.09), **3.77** (2.64, 5.40), **25.9** (19.9, 33.6), and **6.04** (4.90, 7.44), respectively. PRs with same letter were not significantly different.

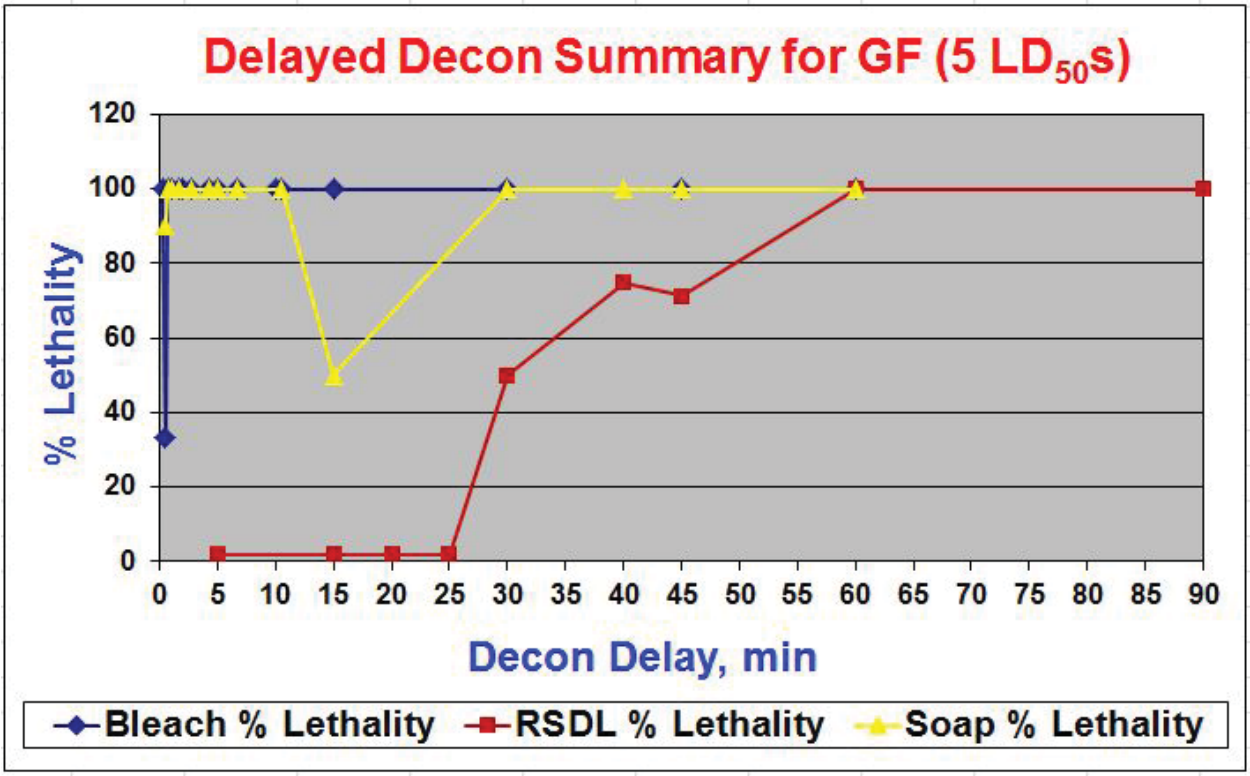


Figure 4. Graph of percent lethality when 0.5% bleach, RSDL, and 1% soapy water decontamination was delayed following challenge by 38.8 mg/kg (5 LD_{50}s) of neat GF. LT_{50} (50% lethality time with 95% CI) values for bleach and RSDL were **0.234*** (0.065, 0.845) and **34.6** (28.3, 41.7) minutes, respectively. *The LT_{50} for bleach was estimated using the less conservative delta method instead of the standard, very conservative Fieller's method. The LT_{50} value for 1% soapy water was estimated to be < 0.5 minutes by using a sequential limit test and observing 9 out of 10 deaths at a delay time of 0.5 minutes. The dose-response curve slopes (95% CI) for 0.5% bleach and RSDL were **2.04** (-0.270, 4.34) and **8.28** (3.07, 13.5), respectively.

APPENDIX A: EXPERIMENTAL RAW DATA

Agent	Date	Treat-ment	Dose mg/kg	Log Dose	Number Animals	Number Dead	Time to Death (hr)
GF IPA Sol'n	18-Jul-06	Control	0.050	-1.301	1	0	>24
GF IPA Sol'n	18-Jul-06	Control	0.500	-0.301	1	0	>24
GF Neat	25-Jul-06	Control	0.500	-0.301	1	0	>24
GF Neat	25-Jul-06	Control	1.000	0.000	1	0	>24
GF IPA Sol'n	18-Jul-06	Control	5.00	0.699	1	0	>24
GF Neat	25-Jul-06	Control	5.00	0.699	1	0	>24
GF Neat	16-Aug-06	Control	6.16	0.790	2	0	>24
GF Neat	22-Aug-06	Control	6.16	0.790	2	2	1, 4
GF Neat	27-Jul-06	Control	6.90	0.839	1	0	>24
GF Neat	01-Aug-06	Control	7.70	0.886	2	0	>24
GF Neat	03-Aug-06	Control	7.70	0.886	2	1	O/N, >24
GF Neat	10-Aug-06	Control	8.10	0.908	2	1	4, >24
GF Neat	01-Aug-06	Control	8.50	0.929	2	1	O/N, >24
GF Neat	03-Aug-06	Control	8.50	0.929	2	1	O/N, >24
GF Neat	10-Aug-06	Control	8.50	0.929	2	2	3, O/N
GF Neat	27-Jul-06	Control	9.50	0.978	1	1	2
GF Neat	22-Aug-06	Control	9.50	0.978	2	1	2, >24
GF Neat	16-Aug-06	Control	10.7	1.029	2	2	3, 3
GF Neat	27-Jul-06	Control	13.2	1.121	1	1	O/N
GF Neat	27-Jul-06	Control	18.2	1.260	1	1	2
GF IPA Sol'n	18-Jul-06	Control	25.0	1.398	1	1	1
GF Neat	25-Jul-06	Control	25.0	1.398	1	1	2

Table A1. Raw data for positive control animals challenged with neat and 5% GF IPA solution in the standard 2-minute decontamination product experiments. O/N = overnight (6-20 hours). These same animals were used for the SERPACWA experiments. The SAS analysis only used animals challenged with neat GF.

Agent	Date	Treat-ment	Dose mg/kg	Log Dose	Number Animals	Number Dead	Time to Death (hr)
GF Neat	18-Jul-06	Bleach	1.00	0.000	1	0	>24
GF Neat	18-Jul-06	Bleach	10.00	1.000	1	0	>24
GF Neat	25-Jul-06	Bleach	17.80	1.250	1	0	>24
GF Neat	25-Jul-06	Bleach	31.6	1.500	1	0	>24
GF Neat	03-Aug-06	Bleach	31.6	1.500	1	0	>24
GF Neat	10-Aug-06	Bleach	31.6	1.500	1	0	>24
GF Neat	03-Aug-06	Bleach	33.5	1.525	1	0	>24
GF Neat	10-Aug-06	Bleach	33.5	1.525	1	0	>24
GF Neat	22-Aug-06	Bleach	33.5	1.525	2	0	>24
GF Neat	27-Jul-06	Bleach	35.5	1.550	1	1	O/N
GF Neat	03-Aug-06	Bleach	35.5	1.550	1	0	>24
GF Neat	10-Aug-06	Bleach	35.5	1.550	1	0	>24
GF Neat	27-Jul-06	Bleach	39.8	1.600	1	1	1
GF Neat	16-Aug-06	Bleach	39.8	1.600	3	0	>24
GF Neat	27-Jul-06	Bleach	44.7	1.650	1	1	1
GF Neat	22-Aug-06	Bleach	44.7	1.650	1	0	>24
GF Neat	24-Aug-06	Bleach	44.7	1.650	2	0	>24
GF Neat	25-Jul-06	Bleach	56.0	1.748	1	1	2
GF Neat	24-Aug-06	Bleach	56.0	1.748	2	0	>24
GF Neat	24-Aug-06	Bleach	79.4	1.900	3	3	1, 1, 1
GF Neat	18-Jul-06	Bleach	100	2.000	1	1	1

Table A2. Raw data for 0.5% bleach animals challenged with neat GF in the standard 2-minute decontamination product experiments. O/N = overnight (6-20 hours).

Agent	Date	Treat-ment	Dose mg/kg	Log Dose	Number Animals	Number Dead	Time to Death (hr)
GF Neat	18-Jul-06	M291	0.200	-0.699	1	0	>24
GF Neat	18-Jul-06	M291	2.00	0.301	1	0	>24
GF Neat	25-Jul-06	M291	3.54	0.549	1	0	>24
GF Neat	25-Jul-06	M291	6.30	0.799	1	0	>24
GF Neat	25-Jul-06	M291	11.2	1.049	1	0	>24
GF Neat	29-Aug-06	M291	11.2	1.049	2	0	>24
GF Neat	27-Jul-06	M291	13.0	1.114	1	0	>24
GF Neat	27-Jul-06	M291	15.0	1.176	1	0	>24
GF Neat	29-Aug-06	M291	15.0	1.176	2	0	>24
GF Neat	27-Jul-06	M291	17.3	1.238	1	0	>24
GF Neat	03-Aug-06	M291	18.6	1.270	1	0	>24
GF Neat	24-Aug-06	M291	18.6	1.270	2	1	2, >24
GF Neat	18-Jul-06	M291	20.0	1.301	1	1	1
GF Neat	03-Aug-06	M291	25.1	1.400	1	0	>24
GF Neat	03-Aug-06	M291	31.6	1.500	1	1	2
GF Neat	10-Aug-06	M291	31.6	1.500	1	1	2
GF Neat	24-Aug-06	M291	31.6	1.500	2	1	1, >24
GF Neat	10-Aug-06	M291	39.8	1.600	1	0	>24
GF Neat	10-Aug-06	M291	50.1	1.700	1	0	>24
GF Neat	16-Aug-06	M291	50.1	1.700	1	1	1
GF Neat	22-Aug-06	M291	50.1	1.700	2	2	1, 1
GF Neat	24-Aug-06	M291	63.0	1.799	2	1	1, 1
GF Neat	16-Aug-06	M291	79.4	1.900	1	1	1
GF Neat	22-Aug-06	M291	79.4	1.900	1	1	1
GF Neat	29-Aug-06	M291	79.4	1.900	2	2	1, 1
GF Neat	29-Aug-06	M291	100	2.000	2	2	1, 1
GF Neat	16-Aug-06	M291	126	2.100	1	1	1

Table A3. Raw data for M291 SDK animals challenged with neat GF in the standard 2-minute decontamination product experiments. O/N = overnight (6-20 hours).

Agent	Date	Treat-ment	Dose mg/kg	Log Dose	Number Animals	Number Dead	Time to Death (hr)
GF Neat	18-Jul-06	RSDL	5.00	0.699	1	0	>24
GF Neat	18-Jul-06	RSDL	50.0	1.699	1	0	>24
GF Neat	27-Jul-06	RSDL	70.8	1.850	1	0	>24
GF Neat	27-Jul-06	RSDL	100	2.000	1	0	>24
GF Neat	29-Aug-06	RSDL	100	2.000	2	0	>24
GF Neat	27-Jul-06	RSDL	141	2.149	1	0	>24
GF Neat	29-Aug-06	RSDL	141	2.149	2	0	>24
GF Neat	03-Aug-06	RSDL	168	2.225	1	0	>24
GF Neat	10-Aug-06	RSDL	174	2.241	1	0	>24
GF Neat	16-Aug-06	RSDL	174	2.241	3	2	2, O/N, >24
GF Neat	10-Aug-06	RSDL	186	2.270	1	0	2
GF Neat	18-Jul-06	RSDL	200	2.301	1	0	>24
GF Neat	25-Jul-06	RSDL	200	2.301	1	1	2
GF Neat	03-Aug-06	RSDL	200	2.301	1	1	2
GF Neat	10-Aug-06	RSDL	200	2.301	1	0	>24
GF Neat	03-Aug-06	RSDL	224	2.350	1	0	O/N
GF Neat	25-Jul-06	RSDL	245	2.389	1	1	1
GF Neat	22-Aug-06	RSDL	245	2.389	2	1	1, >24
GF Neat	25-Jul-06	RSDL	300	2.477	1	1	2
GF Neat	22-Aug-06	RSDL	300	2.477	1	1	3
GF Neat	24-Aug-06	RSDL	300	2.477	2	1	1, >24
GF Neat	24-Aug-06	RSDL	354	2.549	1	1	O/N
GF Neat	29-Aug-06	RSDL	354	2.549	2	2	4, 4
GF Neat	29-Aug-06	RSDL	457	2.660	2	2	1, 1

Table A4. Raw data for RSDL animals challenged with neat GF in the standard 2-minute decontamination product experiments. O/N = overnight (6-20 hours).

Agent	Date	Treat-ment	Dose mg/kg	Log Dose	Number Animals	Number Dead	Time to Death (hr)
GF Neat	18-Jul-06	Soap	1.00	0.000	1	0	>24
GF Neat	18-Jul-06	Soap	10.0	1.000	1	0	>24
GF Neat	25-Jul-06	Soap	17.8	1.250	1	0	>24
GF Neat	25-Jul-06	Soap	31.6	1.500	1	0	>24
GF Neat	27-Jul-06	Soap	36.5	1.562	1	0	>24
GF Neat	27-Jul-06	Soap	42.2	1.625	1	0	>24
GF Neat	22-Aug-06	Soap	42.2	1.625	2	1	1, >24
GF Neat	27-Jul-06	Soap	48.7	1.688	1	0	>24
GF Neat	03-Aug-06	Soap	48.7	1.688	1	0	>24
GF Neat	10-Aug-06	Soap	48.7	1.688	1	1	1
GF Neat	10-Aug-06	Soap	50.6	1.704	1	1	1
GF Neat	16-Aug-06	Soap	50.6	1.704	2	2	O/N, O/N
GF Neat	03-Aug-06	Soap	52.5	1.720	1	1	2
GF Neat	10-Aug-06	Soap	52.5	1.720	1	1	2
GF Neat	22-Aug-06	Soap	52.5	1.720	1	0	>24
GF Neat	25-Jul-06	Soap	56.0	1.748	1	1	1
GF Neat	03-Aug-06	Soap	56.0	1.748	1	1	2
GF Neat	16-Aug-06	Soap	56.0	1.748	1	1	1
GF Neat	18-Jul-06	Soap	100	2.000	1	1	1

Table A5. Raw data for 1% soapy water animals challenged with neat GF in the standard 2-minute decontamination product experiments. O/N = overnight (6-20 hours).

Agent	Date	Treatment	Dose mg/kg	Log Dose	Number Animals	Number Dead	Time to Death (hr)
GF Neat	01-Aug-06	SERPACWA	0.500	-0.301	2	0	>24
GF Neat	01-Aug-06	SERPACWA	1.00	0.000	2	0	>24
GF Neat	01-Aug-06	SERPACWA	5.00	0.699	2	0	>24
GF Neat	01-Aug-06	SERPACWA	25.0	1.398	2	1	4, >24
GF Neat	08-Aug-06	SERPACWA	25.0	1.398	3	0	>24
GF Neat	01-Aug-06	SERPACWA	126	2.100	2	0	>24
GF Neat	08-Aug-06	SERPACWA	225	2.352	4	0	>24
GF Neat	01-Aug-06	SERPACWA	250	2.398	1	0	>24
GF Neat	08-Aug-06	SERPACWA	250	2.398	4	0	>24
GF Neat	23-Aug-06	SERPACWA	250	2.398	5	1	3, >24, >24, >24, >24
GF Neat	08-Aug-06	SERPACWA	NA	NA	3	0	>24

Table A6. Raw data for SERPACWA animals challenged with neat GF in the SERPACWA experiments. The positive control animals used to calculate the PR for SERPACWA were same animals used for the 2-minute decontamination experiments. NA = not applicable. O/N = overnight (6-20 hours).

Agent	Date	Treatment	Time Delay, min	Number Animals	Number Dead	Time to Death (hr)
GF Neat	02-Sep-10	Bleach delayed	0.25	3	3	1, 1, 1
GF Neat	24-Aug-10	Bleach delayed	0.5	1	1	1
GF Neat	26-Aug-10	Bleach delayed	0.5	5	1	1, >24, >24, >24, >24
GF Neat	24-Aug-10	Bleach delayed	0.67	1	1	1
GF Neat	24-Aug-10	Bleach delayed	1	1	1	1
GF Neat	02-Sep-10	Bleach delayed	1	3	3	1, 1, 1
GF Neat	14-Sep-10	Bleach delayed	1	2	2	1, 1
GF Neat	24-Aug-10	Bleach delayed	1.67	1	1	1
GF Neat	19-Aug-10	Bleach delayed	2	1	1	1
GF Neat	02-Sep-10	Bleach delayed	2	3	3	1, 1, 1
GF Neat	24-Aug-10	Bleach delayed	2.67	1	1	1
GF Neat	24-Aug-10	Bleach delayed	4.17	1	1	1
GF Neat	17-Aug-10	Bleach delayed	5	1	1	1
GF Neat	19-Aug-10	Bleach delayed	5	2	2	1, 2
GF Neat	24-Aug-10	Bleach delayed	6.67	1	1	1
GF Neat	19-Aug-10	Bleach delayed	10	2	2	1, 2
GF Neat	24-Aug-10	Bleach delayed	10.5	1	1	1
GF Neat	17-Aug-10	Bleach delayed	15	2	2	1, 1
GF Neat	19-Aug-10	Bleach delayed	15	2	2	1, 1
GF Neat	17-Aug-10	Bleach delayed	30	2	2	1, 1
GF Neat	19-Aug-10	Bleach delayed	30	1	1	1
GF Neat	17-Aug-10	Bleach delayed	45	2	2	1, 1
GF Neat	17-Aug-10	Bleach delayed	60	1	1	2

Table A7. Raw data for delayed decontamination for 0.5% bleach animals challenged with 38.8 mg/kg BW neat GF (5 LD_{50}s). NA = not applicable. O/N = overnight (6-20 hours).

Agent	Date	Treatment	Time Delay, min	Number Animals	Number Dead	Time to Death (hr)
GF Neat	26-Aug-10	RSDL delayed	0.5	1	0	>24
GF Neat	28-Jul-10	RSDL delayed	5	1	0	>24
GF Neat	05-Aug-10	RSDL delayed	5	2	0	>24
GF Neat	28-Jul-10	RSDL delayed	15	1	0	>24
GF Neat	03-Aug-10	RSDL delayed	15	1	0	>24
GF Neat	05-Aug-10	RSDL delayed	15	2	0	>24
GF Neat	05-Aug-10	RSDL delayed	20	4	0	>24
GF Neat	05-Aug-10	RSDL delayed	25	4	0	>24
GF Neat	28-Jul-10	RSDL delayed	30	1	1	O/N
GF Neat	03-Aug-10	RSDL delayed	30	5	2	1, 1, >24, >24, >24
GF Neat	05-Aug-10	RSDL delayed	40	4	3	1, 2, 2, >24
GF Neat	28-Jul-10	RSDL delayed	45	1	0	>24
GF Neat	03-Aug-10	RSDL delayed	45	6	5	2, 2, 2, 2, 3, >24
GF Neat	28-Jul-10	RSDL delayed	60	1	1	2
GF Neat	03-Aug-10	RSDL delayed	60	4	4	1, 2, 2, 2
GF Neat	28-Jul-10	RSDL delayed	90	1	1	1

Table A8. Raw data for delayed decontamination for RSDL animals challenged with 38.8 mg/kg BW neat GF (5 LD_{50}s). NA = not applicable. O/N = overnight (6-20 hours).

Agent	Date	Treatment	Time Delay, min	Number Animals	Number Dead	Time to Death (hr)
GF Neat	24-Aug-10	Soap delayed	0.5	1	1	1
GF Neat	26-Aug-10	Soap delayed	0.5	5	4	1, 1, 2, O/N, >24
GF Neat	02-Sep-10	Soap delayed	0.5	4	4	1, 1, 1, 1
GF Neat	24-Aug-10	Soap delayed	0.67	1	1	1
GF Neat	24-Aug-10	Soap delayed	1	1	1	1
GF Neat	24-Aug-10	Soap delayed	1.67	1	1	1
GF Neat	24-Aug-10	Soap delayed	2.67	1	1	1
GF Neat	24-Aug-10	Soap delayed	4.17	1	1	1
GF Neat	17-Aug-10	Soap delayed	5	1	1	1
GF Neat	24-Aug-10	Soap delayed	6.67	1	1	1
GF Neat	24-Aug-10	Soap delayed	10.5	1	1	1
GF Neat	17-Aug-10	Soap delayed	15	2	1	1, >24
GF Neat	17-Aug-10	Soap delayed	30	2	2	1, 2
GF Neat	19-Aug-10	Soap delayed	30	1	1	2
GF Neat	19-Aug-10	Soap delayed	40	1	1	1
GF Neat	17-Aug-10	Soap delayed	45	2	2	1, 2
GF Neat	17-Aug-10	Soap delayed	60	1	1	2

Table A9. Raw data for delayed decontamination for 1% soapy water animals challenged with 38.8 mg/kg BW neat GF (5 LD_{50}s). NA = not applicable. O/N = overnight (6-20 hours).

APENDIX B: SAS OUTPUT FILES

SAS ANALYSIS USING PROBSEPX AND PRORATIO PROGRAMS FOR DECON GF LD_{50}
Final Report After complete QC Audit of Data

File: GF decon SAS output including 95 and 99.5 % CI 150630.doc

```
                                    LD50 GF DECON                                1
                                                  10:27 Tuesday, June 30, 2015
                     SEPARATE-SLOPES DOSE-RESPONSE FITS
        PROBIT FITS TO DATA USING ALL EXPERIMENTAL STAGES, LOG10(DOSE)

------------------------- AGENT=GF TRTGRP=Acontrol -------------------------

     Non-Linear Least Squares Grid Search     Dependent Variable NDEAD
                         B1             B01       Weighted loss
                         2.283362       3.182352      31.346001

                   Non-Linear Least Squares Iterative Phase
               Dependent Variable NDEAD   Method: Gauss-Newton
              Iter       B1             B01       Weighted loss
                 0       2.283362       3.182352      31.346001
                 1       3.855537       1.643104      29.546024
                 2       5.850239      -0.187583      28.685072
                 3       6.779849      -1.016181      28.578175
                 4       6.931492      -1.149117      28.575713
                 5       6.940654      -1.156891      28.575701
                 6       6.941186      -1.157345      28.575701
NOTE: Convergence criterion met.


   Non-Linear Least Squares Summary Statistics     Dependent Variable NDEAD

       Source                DF    Weighted SS     Weighted MS

       Regression             2   5131.9838779    2565.9919390
       Residual              16     12.2426456       0.7651653
       Uncorrected Total     18   5144.2265235

       (Corrected Total)     17   5016.0723840
       Sum of Loss                  28.5757006

       Parameter     Estimate    Asymptotic             Asymptotic 95 %
                                 Std. Error         Confidence Interval
                                                    Lower         Upper
       B1         6.941186457  3.3800218640 -0.2241041949  14.106477110
       B01       -1.157344608  3.0675398177 -7.6602062067   5.345516990


                       Asymptotic Correlation Matrix

                   Corr                B1               B01
                   ffffffffffffffffffffffffffffffffffffffff
                   B1                   1      -0.996031509
                   B01       -0.996031509                 1
```

SEPARATE-SLOPES DOSE-RESPONSE FITS
PROBIT FITS TO DATA USING ALL EXPERIMENTAL STAGES, LOG10(DOSE)

---------- AGENT=GF TRTGRP=Bleach ----------

Non-Linear Least Squares Grid Search Dependent Variable NDEAD

B1	B01	Weighted loss
1.485999	2.064495	27.306000

Non-Linear Least Squares Iterative Phase
Dependent Variable NDEAD Method: Gauss-Newton

Iter	B1	B01	Weighted loss
0	1.485999	2.064495	27.306000
1	4.266027	-2.499767	21.908702
2	6.391304	-6.072294	20.389535
3	7.247903	-7.494594	20.239814
4	7.423557	-7.780585	20.234510
5	7.443874	-7.813296	20.234436
6	7.445872	-7.816513	20.234435
7	7.446063	-7.816820	20.234435

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable NDEAD

Source	DF	Weighted SS	Weighted MS
Regression	2	94.05967894	47.02983947
Residual	19	22.12073441	1.16424918
Uncorrected Total	21	116.18041335	
(Corrected Total)	20	116.13874420	
Sum of Loss		20.23443510	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
B1	7.446062643	2.6601917229	1.878257163	13.013868124
B01	-7.816819568	4.4087574075	-17.044388936	1.410749801

Asymptotic Correlation Matrix

Corr	B1	B01
B1	1	-0.997420017
B01	-0.997420017	1

SEPARATE-SLOPES DOSE-RESPONSE FITS
PROBIT FITS TO DATA USING ALL EXPERIMENTAL STAGES, LOG10(DOSE)

---------- AGENT=GF TRTGRP=M291 ----------

Non-Linear Least Squares Grid Search Dependent Variable NDEAD

B1	B01	Weighted loss
1.718265	2.539581	29.025831

Non-Linear Least Squares Iterative Phase
Dependent Variable NDEAD Method: Gauss-Newton

Iter	B1	B01	Weighted loss
0	1.718265	2.539581	29.025831
1	3.053066	0.555264	23.731065
2	3.976270	-0.818791	22.481035
3	4.355412	-1.375547	22.353446
4	4.425018	-1.477687	22.350009
5	4.430709	-1.486205	22.349985
6	4.431078	-1.486760	22.349985

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable NDEAD

Source	DF	Weighted SS	Weighted MS
Regression	2	855.02922302	427.51461151
Residual	25	16.81140461	0.67245618
Uncorrected Total	27	871.84062763	
(Corrected Total)	26	869.72818646	
Sum of Loss		22.34998477	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
B1	4.431078075	1.2568526970	1.8425622077	7.0195939420
B01	-1.486760266	1.8617422272	-5.3210594700	2.3475389380

Asymptotic Correlation Matrix

Corr	B1	B01
B1	1	-0.986682163
B01	-0.986682163	1

SEPARATE-SLOPES DOSE-RESPONSE FITS
PROBIT FITS TO DATA USING ALL EXPERIMENTAL STAGES, LOG10(DOSE)

---------- AGENT=GF TRTGRP=RSDL ----------

Non-Linear Least Squares Grid Search Dependent Variable NDEAD

B1	B01	Weighted loss
2.290258	-0.112470	31.532930

Non-Linear Least Squares Iterative Phase
Dependent Variable NDEAD Method: Gauss-Newton

Iter	B1	B01	Weighted loss
0	2.290258	-0.112470	31.532930
1	4.509864	-5.297339	26.592113
2	6.073527	-8.941801	25.407259
3	6.804836	-10.644176	25.260948
4	6.949972	-10.983720	25.256379
5	6.960929	-11.009859	25.256345
6	6.961684	-11.011660	25.256345
7	6.961735	-11.011782	25.256345

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable NDEAD

Source	DF	Weighted SS	Weighted MS
Regression	2	447.06751123	223.53375562
Residual	22	13.38806047	0.60854820
Uncorrected Total	24	460.45557171	
(Corrected Total)	23	459.95265129	
Sum of Loss		25.25634454	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
B1	6.96173455	2.3464008008	2.095635886	11.827833219
B01	-11.01178161	5.4269991582	-22.266599349	0.243036138

Asymptotic Correlation Matrix

Corr	B1	B01
B1	1	-0.998601396
B01	-0.998601396	1

SEPARATE-SLOPES DOSE-RESPONSE FITS
PROBIT FITS TO DATA USING ALL EXPERIMENTAL STAGES, LOG10(DOSE)

---------- AGENT=GF TRTGRP=Soap ----------

Non-Linear Least Squares Grid Search Dependent Variable NDEAD

B1	B01	Weighted loss
1.856663	2.123252	22.675908

Non-Linear Least Squares Iterative Phase
Dependent Variable NDEAD Method: Gauss-Newton

Iter	B1	B01	Weighted loss
0	1.856663	2.123252	22.675908
1	4.005592	-1.449470	19.785830
2	7.166867	-6.764344	17.649551
3	12.284671	-15.379010	16.007165
4	16.102900	-21.825423	15.581093
5	17.449043	-24.103721	15.548426
6	17.596498	-24.352050	15.548091
7	17.601791	-24.360807	15.548090
8	17.602047	-24.361232	15.548090

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable NDEAD

Source	DF	Weighted SS	Weighted MS
Regression	2	100040.15600	50020.07800
Residual	17	11.56102	0.68006
Uncorrected Total	19	100051.71702	
(Corrected Total)	18	75075.43026	
Sum of Loss		15.54809	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
B1	17.60204674	8.344756812	-0.003747421	35.207840901
B01	-24.36123212	14.094911387	-54.098720487	5.376256242

Asymptotic Correlation Matrix

Corr	B1	B01
B1	1	-0.999664275
B01	-0.999664275	1

SEPARATE-SLOPES DOSE-RESPONSE FITS
OUTPUT DATA FROM PROC NLIN -- LISTING OF PREDICTED VALUES ANDRESIDUALS

---------- Agent=GF Treatment Group=Acontrol ----------

OBS	STAGE	Agent Dose (mg/kg)	Log10 Dose (mg/kg)	No. Animals	Observed N Dead	Prop. Dead	Probit of Percentile	LCL
1	2	0.50	-.301	1	0	0.000	-1.64485	-0.00000
2	2	1.00	0.000	1	0	0.000	-1.64485	-0.00000
3	2	5.00	0.699	1	0	0.000	-1.64485	-0.17268
4	6	6.16	0.790	2	0	0.000	-1.64485	-0.13801
5	7	6.16	0.790	2	2	1.000	1.64485	-0.13801
6	3	6.90	0.839	1	0	0.000	-1.64485	0.08855
7	.	7.70	0.886	2	0	0.000	-1.64485	0.52437
8	4	7.70	0.886	2	1	0.500	-0.00000	0.52437
9	5	8.10	0.908	2	1	0.500	-0.00000	0.66076
10	.	8.50	0.929	2	1	0.500	-0.00000	0.76750
11	4	8.50	0.929	2	1	0.500	-0.00000	0.76750
12	5	8.50	0.929	2	2	1.000	1.64485	0.76750
13	3	9.50	0.978	1	1	1.000	1.64485	0.47888
14	7	9.50	0.978	2	1	0.500	-0.00000	0.95775
15	6	10.70	1.029	2	2	1.000	1.64485	1.15316
16	3	13.20	1.121	1	1	1.000	1.64485	0.76978
17	3	18.20	1.260	1	1	1.000	1.64485	0.95863
18	2	25.00	1.398	1	1	1.000	1.64485	0.99714

OBS	UCL	Predicted No. Dead	Studentized Residuals	Predicted Prop. Dead	Probit Pred. Pct Dead
1	0.00000	0.00000	-0.00000	0.00000	-1.64485
2	0.00000	0.00000	-0.00000	0.00000	-1.64485
3	0.36435	0.09583	-0.36063	0.09583	-1.30566
4	1.13518	0.49859	-0.93537	0.24929	-0.67672
5	1.13518	0.49859	2.81674	0.24929	-0.67672
6	0.64927	0.36891	-0.79500	0.36891	-0.33474
7	1.46917	0.99677	-1.48532	0.49839	-0.00405
8	1.46917	0.99677	0.00481	0.49839	-0.00405
9	1.57553	1.11815	-0.17681	0.55907	0.14862
10	1.69487	1.23119	-0.35444	0.61559	0.29393
11	1.69487	1.23119	-0.35444	0.61559	0.29393
12	1.69487	1.23119	1.17869	0.61559	0.29393
13	0.99192	0.73540	0.62375	0.73540	0.62922
14	1.98383	1.47079	-0.81878	0.73540	0.62922
15	2.20034	1.67675	0.70537	0.83837	0.98780
16	1.12515	0.94747	0.25405	0.94747	1.62077
17	1.03175	0.99519	0.07172	0.99519	1.64485
18	1.00247	0.99980	0.01369	0.99980	1.64485

SEPARATE-SLOPES DOSE-RESPONSE FITS
OUTPUT DATA FROM PROC NLIN -- LISTING OF PREDICTED VALUES ANDRESIDUALS

---------- Agent=GF Treatment Group=Bleach ----------

OBS	STAGE	Agent Dose (mg/kg)	Log10 Dose (mg/kg)	No. Animals	Observed N Dead	Prop. Dead	Probit of Percentile	LCL
19	1	1.0	0.000	1	0	0.000	-1.64485	-0.00000
20	1	10.0	1.000	1	0	0.000	-1.64485	-0.00000
21	2	17.8	1.250	1	0	0.000	-1.64485	-0.00177
22	2	31.6	1.500	1	0	0.000	-1.64485	-0.06102
23	4	31.6	1.500	1	0	0.000	-1.64485	-0.06102
24	5	31.6	1.500	1	0	0.000	-1.64485	-0.06102
25	4	33.5	1.525	1	0	0.000	-1.64485	-0.06147
26	5	33.5	1.525	1	0	0.000	-1.64485	-0.06147
27	7	33.5	1.525	2	0	0.000	-1.64485	-0.12294
28	3	35.5	1.550	1	1	1.000	1.64485	-0.05379
29	4	35.5	1.550	1	0	0.000	-1.64485	-0.05379
30	5	35.5	1.550	1	0	0.000	-1.64485	-0.05379
31	3	39.8	1.600	1	1	1.000	1.64485	-0.00933
32	6	39.8	1.600	3	0	0.000	-1.64485	-0.02800
33	3	44.7	1.650	1	1	1.000	1.64485	0.06871
34	7	44.7	1.650	1	0	0.000	-1.64485	0.06871
35	8	44.7	1.650	2	0	0.000	-1.64485	0.13742
36	2	56.0	1.748	1	1	1.000	1.64485	0.24774
37	8	56.0	1.748	2	0	0.000	-1.64485	0.49547
38	8	79.4	1.900	3	3	1.000	1.64485	1.96996
39	1	100.0	2.000	1	1	1.000	1.64485	0.88645

OBS	UCL	Predicted No. Dead	Studentized Residuals	Predicted Prop. Dead	Probit Pred. Pct Dead
19	0.00000	0.00000	-0.00000	0.00000	-1.64485
20	0.00000	0.00000	-0.00001	0.00000	-1.64485
21	0.00222	0.00023	-0.01479	0.00023	-1.64485
22	0.15996	0.04947	-0.23517	0.04947	-1.64485
23	0.15996	0.04947	-0.23517	0.04947	-1.64485
24	0.15996	0.04947	-0.23517	0.04947	-1.64485
25	0.20542	0.07197	-0.28735	0.07197	-1.46124
26	0.20542	0.07197	-0.28735	0.07197	-1.46124
27	0.41083	0.14395	-0.42023	0.07197	-1.46124
28	0.25655	0.10138	3.07112	0.10138	-1.27372
29	0.25655	0.10138	-0.34648	0.10138	-1.27372
30	0.25655	0.10138	-0.34648	0.10138	-1.27372
31	0.37534	0.18300	2.17517	0.18300	-0.90399
32	1.12601	0.54900	-0.89947	0.18300	-0.90399
33	0.52842	0.29857	1.57885	0.29857	-0.52853
34	0.52842	0.29857	-0.67204	0.29857	-0.52853
35	1.05684	0.59713	-0.98086	0.29857	-0.52853
36	0.91102	0.57938	0.89963	0.57938	0.20030
37	1.82203	1.15875	-1.86271	0.57938	0.20030
38	3.47886	2.72441	0.79439	0.90814	1.32936
39	1.07559	0.98102	0.14736	0.98102	1.64485

SEPARATE-SLOPES DOSE-RESPONSE FITS
OUTPUT DATA FROM PROC NLIN -- LISTING OF PREDICTED VALUES ANDRESIDUALS

---------- Agent=GF Treatment Group=M291 ----------

OBS	STAGE	Agent Dose (mg/kg)	Log10 Dose (mg/kg)	No. Animals	Observed N Dead	Prop. Dead	Probit of Percentile	LCL
40	1	0.20	-.699	1	0	0.000	-1.64485	-0.00000
41	1	2.00	0.301	1	0	0.000	-1.64485	-0.00000
42	2	3.54	0.549	1	0	0.000	-1.64485	-0.00024
43	2	6.30	0.799	1	0	0.000	-1.64485	-0.00791
44	2	11.20	1.049	1	0	0.000	-1.64485	-0.05808
45	8	11.20	1.049	2	0	0.000	-1.64485	-0.11615
46	3	13.00	1.114	1	0	0.000	-1.64485	-0.07074
47	3	15.00	1.176	1	0	0.000	-1.64485	-0.06993
48	8	15.00	1.176	2	0	0.000	-1.64485	-0.13987
49	3	17.30	1.238	1	0	0.000	-1.64485	-0.04720
50	4	18.60	1.270	1	0	0.000	-1.64485	-0.02503
51	8	18.60	1.270	2	1	0.500	-0.00000	-0.05005
52	1	20.00	1.301	1	1	1.000	1.64485	0.00482
53	4	25.10	1.400	1	0	0.000	-1.64485	0.14128
54	4	31.60	1.500	1	1	1.000	1.64485	0.31421
55	5	31.60	1.500	1	1	1.000	1.64485	0.31421
56	8	31.60	1.500	2	1	0.500	-0.00000	0.62842
57	5	39.80	1.600	1	0	0.000	-1.64485	0.48729
58	5	50.10	1.700	1	0	0.000	-1.64485	0.64946
59	6	50.10	1.700	1	1	1.000	1.64485	0.64946
60	7	50.10	1.700	2	2	1.000	1.64485	1.29891
61	8	63.00	1.799	2	2	1.000	1.64485	1.57884

OBS	UCL	Predicted No. Dead	Studentized Residuals	Predicted Prop. Dead	Probit Pred. Pct Dead
40	0.00000	0.00000	-0.00000	0.00000	-1.64485
41	0.00000	0.00000	-0.00004	0.00000	-1.64485
42	0.00029	0.00003	-0.00424	0.00003	-1.64485
43	0.01114	0.00162	-0.04037	0.00162	-1.64485
44	0.12420	0.03306	-0.19082	0.03306	-1.64485
45	0.24840	0.06612	-0.27914	0.03306	-1.64485
46	0.19169	0.06048	-0.26326	0.06048	-1.55079
47	0.27210	0.10108	-0.34881	0.10108	-1.27541
48	0.54420	0.20216	-0.51490	0.10108	-1.27541
49	0.36408	0.15844	-0.45108	0.15844	-1.00088
50	0.41402	0.19450	-0.51021	0.19450	-0.86145
51	0.82803	0.38899	1.18049	0.19450	-0.86145
52	0.46560	0.23521	1.86933	0.23521	-0.72179
53	0.63460	0.38794	-0.82131	0.38794	-0.28470
54	0.81170	0.56296	0.90842	0.56296	0.15847
55	0.81170	0.56296	0.90842	0.56296	0.15847
56	1.62341	1.12591	-0.19119	0.56296	0.15847
57	0.96583	0.72656	-1.68839	0.72656	0.60245
58	1.05468	0.85207	-2.49769	0.85207	1.04535
59	1.05468	0.85207	0.43363	0.85207	1.04535
60	2.10937	1.70414	0.64047	0.85207	1.04535
61	2.14674	1.86279	0.41598	0.93139	1.48626

---------- Agent=GF Treatment Group=M291 ----------
(continued)

OBS	STAGE	Agent Dose (mg/kg)	Log10 Dose (mg/kg)	No. Animals	Observed N Dead	Prop. Dead	Probit of Percentile	LCL
62	6	79.40	1.900	1	1	1.000	1.64485	0.89332
63	7	79.40	1.900	1	1	1.000	1.64485	0.89332
64	8	79.40	1.900	2	2	1.000	1.64485	1.78665
65	8	100.00	2.000	2	2	1.000	1.64485	1.90995
66	6	125.90	2.100	1	1	1.000	1.64485	0.98432

OBS	UCL	Predicted No. Dead	Studentized Residuals	Predicted Prop. Dead	Probit Pred. Pct Dead
62	1.05325	0.97329	0.17065	0.97329	1.64485
63	1.05325	0.97329	0.17065	0.97329	1.64485
64	2.10651	1.94658	0.24915	0.97329	1.64485
65	2.05499	1.98247	0.13796	0.99123	1.64485
66	1.01086	0.99759	0.04949	0.99759	1.64485

---------- Agent=GF Treatment Group=RSDL ----------

OBS	STAGE	Agent Dose (mg/kg)	Log10 Dose (mg/kg)	No. Animals	Observed N Dead	Prop. Dead	Probit of Percentile	LCL
67	1	5.0	0.699	1	0	0.000	-1.64485	-0.00000
68	1	50.0	1.699	1	0	0.000	-1.64485	-0.00018
69	3	70.8	1.850	1	0	0.000	-1.64485	-0.00597
70	3	100.0	2.000	1	0	0.000	-1.64485	-0.05463
71	8	100.0	2.000	2	0	0.000	-1.64485	-0.10926
72	3	141.0	2.149	1	0	0.000	-1.64485	-0.07879
73	8	141.0	2.149	2	0	0.000	-1.64485	-0.15758
74	4	168.0	2.225	1	0	0.000	-1.64485	0.04968
75	5	174.0	2.241	1	0	0.000	-1.64485	0.08926
76	6	174.0	2.241	3	2	0.667	0.43073	0.26777

OBS	UCL	Predicted No. Dead	Studentized Residuals	Predicted Prop. Dead	Probit Pred. Pct Dead
67	0.00000	0.00000	-0.00000	0.00000	-1.64485
68	0.00021	0.00001	-0.00290	0.00001	-1.64485
69	0.00770	0.00087	-0.02947	0.00087	-1.64485
70	0.09140	0.01838	-0.14177	0.01838	-1.64485
71	0.18280	0.03677	-0.20835	0.01838	-1.64485
72	0.37274	0.14698	-0.43620	0.14698	-1.04949
73	0.74549	0.29395	-0.65185	0.14698	-1.04949
74	0.55354	0.30161	-0.68145	0.30161	-0.51977
75	0.58986	0.33956	-0.74150	0.33956	-0.41367
76	1.76957	1.01867	1.33334	0.33956	-0.41367

---------- Agent=GF Treatment Group=RSDL ----------
(continued)

OBS	STAGE	Agent Dose (mg/kg)	Log10 Dose (mg/kg)	No. Animals	Observed N Dead	Prop. Dead	Probit of Percentile	LCL
77	5	186	2.270	1	1	1.000	1.64485	0.17176
78	1	200	2.301	1	0	0.000	-1.64485	0.26499
79	2	200	2.301	1	1	1.000	1.64485	0.26499
80	4	200	2.301	1	1	1.000	1.64485	0.26499
81	5	200	2.301	1	0	0.000	-1.64485	0.26499
82	4	224	2.350	1	1	1.000	1.64485	0.40161
83	2	245	2.389	1	1	1.000	1.64485	0.49922
84	7	245	2.389	2	1	0.500	-0.00000	0.99843
85	2	300	2.477	1	1	1.000	1.64485	0.70313
86	7	300	2.477	1	1	1.000	1.64485	0.70313
87	8	300	2.477	2	1	0.500	-0.00000	1.40625
88	8	354	2.549	1	1	1.000	1.64485	0.84244
89	8	354	2.549	2	2	1.000	1.64485	1.68488
90	8	457	2.660	2	2	1.000	1.64485	1.92542

OBS	UCL	Predicted No. Dead	Studentized Residuals	Predicted Prop. Dead	Probit Pred. Pct Dead
77	0.66032	0.41604	1.22007	0.41604	-0.21203
78	0.74089	0.50294	-1.03346	0.50294	0.00738
79	0.74089	0.50294	1.02137	0.50294	0.00738
80	0.74089	0.50294	1.02137	0.50294	0.00738
81	0.74089	0.50294	-1.03346	0.50294	0.00738
82	0.87207	0.63684	0.77706	0.63684	0.35002
83	0.96616	0.73269	0.62455	0.73269	0.62096
84	1.93232	1.46537	-0.79690	0.73269	0.62096
85	1.07940	0.89126	0.36511	0.89126	1.23328
86	1.07940	0.89126	0.36511	0.89126	1.23328
87	2.15880	1.78253	-1.95075	0.89126	1.23328
88	1.07459	0.95851	0.21672	0.95851	1.64485
89	2.14918	1.91703	0.32052	0.95851	1.64485
90	2.05014	1.98778	0.11519	0.99389	1.64485

---------- Agent=GF Treatment Group=Soap ----------

OBS	STAGE	Agent Dose (mg/kg)	Log10 Dose (mg/kg)	No. Animals	Observed N Dead	Prop. Dead	Probit of Percentile	LCL
91	1	1	0.000	1	0	0.000	-1.64485	-7.4982E-187

OBS	UCL	Predicted No. Dead	Studentized Residuals	Predicted Prop. Dead	Probit Pred. Pct Dead
91	7.5154E-187	8.5885E-190	-2.7159E-187	8.5885E-190	-1.64485

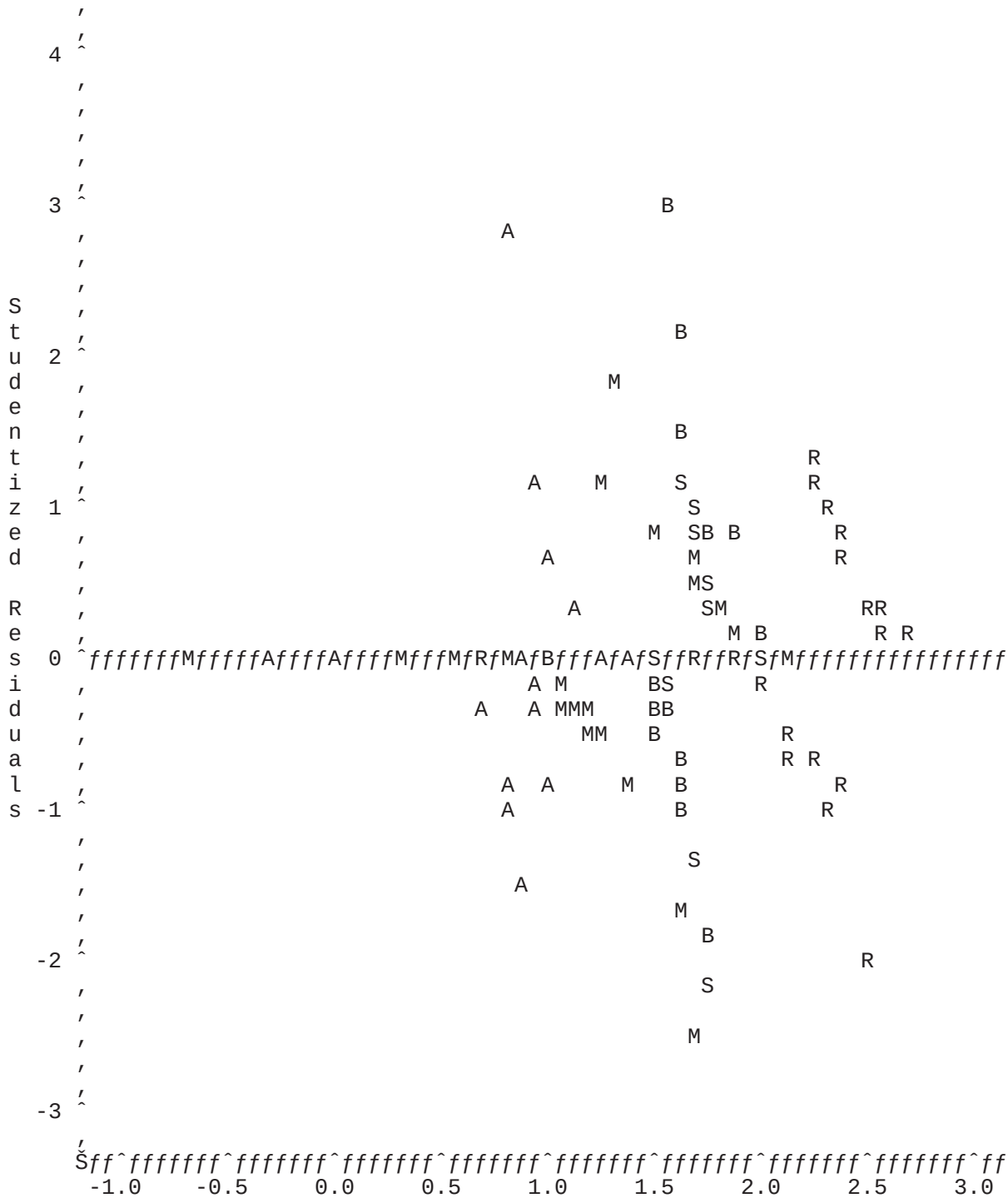
---------- Agent=GF Treatment Group=Soap ----------

(continued)

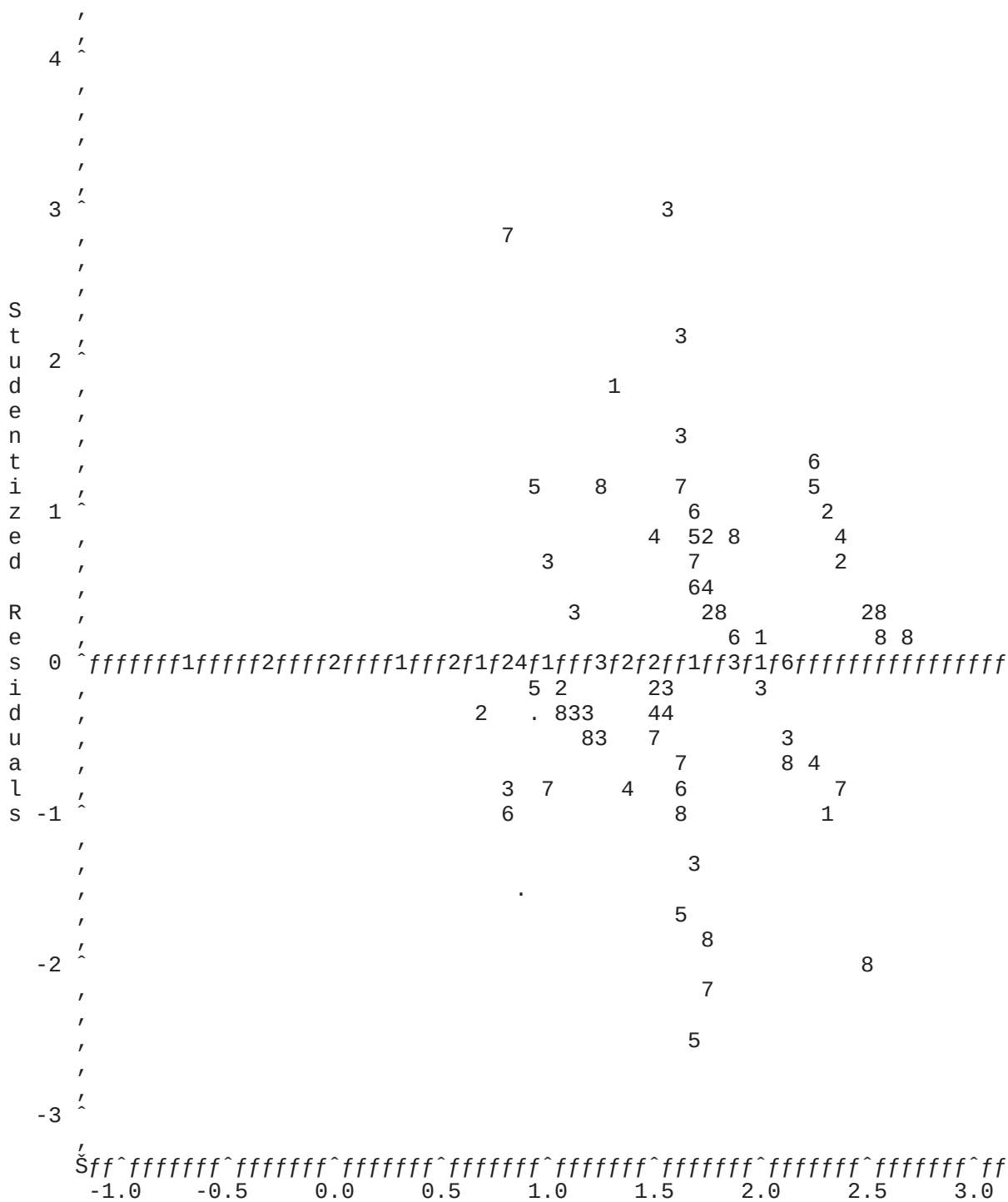
OBS	STAGE	Agent Dose (mg/kg)	Log10 Dose (mg/kg)	No. Animals	Observed N Dead	Prop. Dead	Probit of Percentile	LCL
92	1	10.0	1.000	1	0	0.000	-1.64485	-0.00000
93	2	17.8	1.250	1	0	0.000	-1.64485	-0.00000
94	2	31.6	1.500	1	0	0.000	-1.64485	-0.01533
95	3	36.5	1.562	1	0	0.000	-1.64485	-0.13451
96	3	42.2	1.625	1	0	0.000	-1.64485	-0.18086
97	7	42.2	1.625	2	1	0.500	-0.00000	-0.36172
98	3	48.7	1.688	1	0	0.000	-1.64485	0.34420
99	4	48.7	1.688	1	0	0.000	-1.64485	0.34420
100	5	48.7	1.688	1	1	1.000	1.64485	0.34420
101	5	50.6	1.704	1	1	1.000	1.64485	0.47063
102	6	50.6	1.704	2	2	1.000	1.64485	0.94127
103	4	52.5	1.720	1	1	1.000	1.64485	0.57135
104	5	52.5	1.720	1	1	1.000	1.64485	0.57135
105	7	52.5	1.720	1	0	0.000	-1.64485	0.57135
106	2	56.0	1.748	1	1	1.000	1.64485	0.72857
107	4	56.0	1.748	1	1	1.000	1.64485	0.72857
108	6	56.0	1.748	1	1	1.000	1.64485	0.72857
109	1	100.0	2.000	1	1	1.000	1.64485	1.00000

OBS	UCL	Predicted No. Dead	Studentized Residuals	Predicted Prop. Dead	Probit Pred. Pct Dead
92	0.00000	0.00000	-0.00000	0.00000	-1.64485
93	0.00000	0.00000	-0.00000	0.00000	-1.64485
94	0.01837	0.00152	-0.03973	0.00152	-1.64485
95	0.19716	0.03132	-0.20147	0.03132	-1.64485
96	0.63267	0.22590	-0.60875	0.22590	-0.75241
97	1.26534	0.45181	1.22253	0.22590	-0.75241
98	0.92400	0.63410	-1.37346	0.63410	0.34273
99	0.92400	0.63410	-1.37346	0.63410	0.34273
100	0.92400	0.63410	0.79254	0.63410	0.34273
101	1.00414	0.73739	0.62303	0.73739	0.63530
102	2.00828	1.47477	0.92364	0.73739	0.63530
103	1.06955	0.82045	0.49162	0.82045	0.91709
104	1.06955	0.82045	0.49162	0.82045	0.91709
105	1.06955	0.82045	-2.24651	0.82045	0.91709
106	1.11302	0.92080	0.31153	0.92080	1.41046
107	1.11302	0.92080	0.31153	0.92080	1.41046
108	1.11302	0.92080	0.31153	0.92080	1.41046
109	1.00000	1.00000	0.00000	1.00000	1.64485

SEPARATE-SLOPES DOSE-RESPONSE FITS
OUTPUT DATA FROM PROC NLIN -- LISTING OF PREDICTED VALUES ANDRESIDUALS
RESIDUALS VS. LOGDOSE - PLOTTING SYMBOL IS TREATMENT GROUP



SEPARATE-SLOPES DOSE-RESPONSE FITS
OUTPUT DATA FROM PROC NLIN -- LISTING OF PREDICTED VALUES ANDRESIDUALS
RESIDUALS VS. LOGDOSE - PLOTTING SYMBOL IS EXPERIMENTAL STAGE



SEPARATE-SLOPES DOSE-RESPONSE FITS
OUTPUT DATA FROM PROC NLIN -- LISTING OF PREDICTED VALUES ANDRESIDUALS
DESCRIPTIVE STATISTICS OF RESIDUALS BY STAGE

Analysis Variable : STUDRES Studentized Residuals

---------- Agent=GF Experimental Stage=. ----------

Mean	N	Std Dev	Std Error	Minimum	Maximum
-0.9198806	2	0.7996587	0.5654441	-1.4853247	-0.3544365

---------- Agent=GF Experimental Stage=1 ----------

Mean	N	Std Dev	Std Error	Minimum	Maximum
0.0816896	12	0.6398929	0.1847212	-1.0334624	1.8693320

---------- Agent=GF Experimental Stage=2 ----------

Mean	N	Std Dev	Std Error	Minimum	Maximum
0.1468831	16	0.3960773	0.0990193	-0.3606340	1.0213655

---------- Agent=GF Experimental Stage=3 ----------

Mean	N	Std Dev	Std Error	Minimum	Maximum
0.1953377	16	1.1558387	0.2889597	-1.3734646	3.0711199

---------- Agent=GF Experimental Stage=4 ----------

Mean	N	Std Dev	Std Error	Minimum	Maximum
-0.0782193	14	0.7013471	0.1874429	-1.3734646	1.0213655

---------- Agent=GF Experimental Stage=5 ----------

Mean	N	Std Dev	Std Error	Minimum	Maximum
-0.1280347	14	1.0999222	0.2939666	-2.4976944	1.2200672

SEPARATE-SLOPES DOSE-RESPONSE FITS
OUTPUT DATA FROM PROC NLIN -- LISTING OF PREDICTED VALUES ANDRESIDUALS
DESCRIPTIVE STATISTICS OF RESIDUALS BY STAGE

Analysis Variable : STUDRES Studentized Residuals

---------- Agent=GF Experimental Stage=6 ----------

Mean	N	Std Dev	Std Error	Minimum	Maximum
0.2325349	9	0.7617762	0.2539254	-0.9353731	1.3333414

---------- Agent=GF Experimental Stage=7 ----------

Mean	N	Std Dev	Std Error	Minimum	Maximum
0.0261039	10	1.3733530	0.4342924	-2.2465071	2.8167369

---------- Agent=GF Experimental Stage=8 ----------

Mean	N	Std Dev	Std Error	Minimum	Maximum
-0.2005835	16	0.8517861	0.2129465	-1.9507452	1.1804881

SEPARATE-SLOPES DOSE-RESPONSE FITS
OUTPUT DATA FROM PROC NLIN -- LISTING OF PREDICTED VALUES ANDRESIDUALS
ANOVA OF RESIDUALS FOR STAGE EFFECTS

---------- Agent=GF ----------

General Linear Models Procedure
Class Level Information

Class	Levels	Values
STAGE	8	1 2 3 4 5 6 7 8

Number of observations in by group = 109

NOTE: Due to missing values, only 107 observations can be used in this analysis.

SEPARATE-SLOPES DOSE-RESPONSE FITS
OUTPUT DATA FROM PROC NLIN -- LISTING OF PREDICTED VALUES ANDRESIDUALS
ANOVA OF RESIDUALS FOR STAGE EFFECTS

---------- Agent=GF ----------

General Linear Models Procedure

Dependent Variable: STUDRES Studentized Residuals

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	2.52332051	0.31541506	0.38	0.9291
Error	98	81.41546218	0.83077002		
Corrected Total	106	83.93878269			

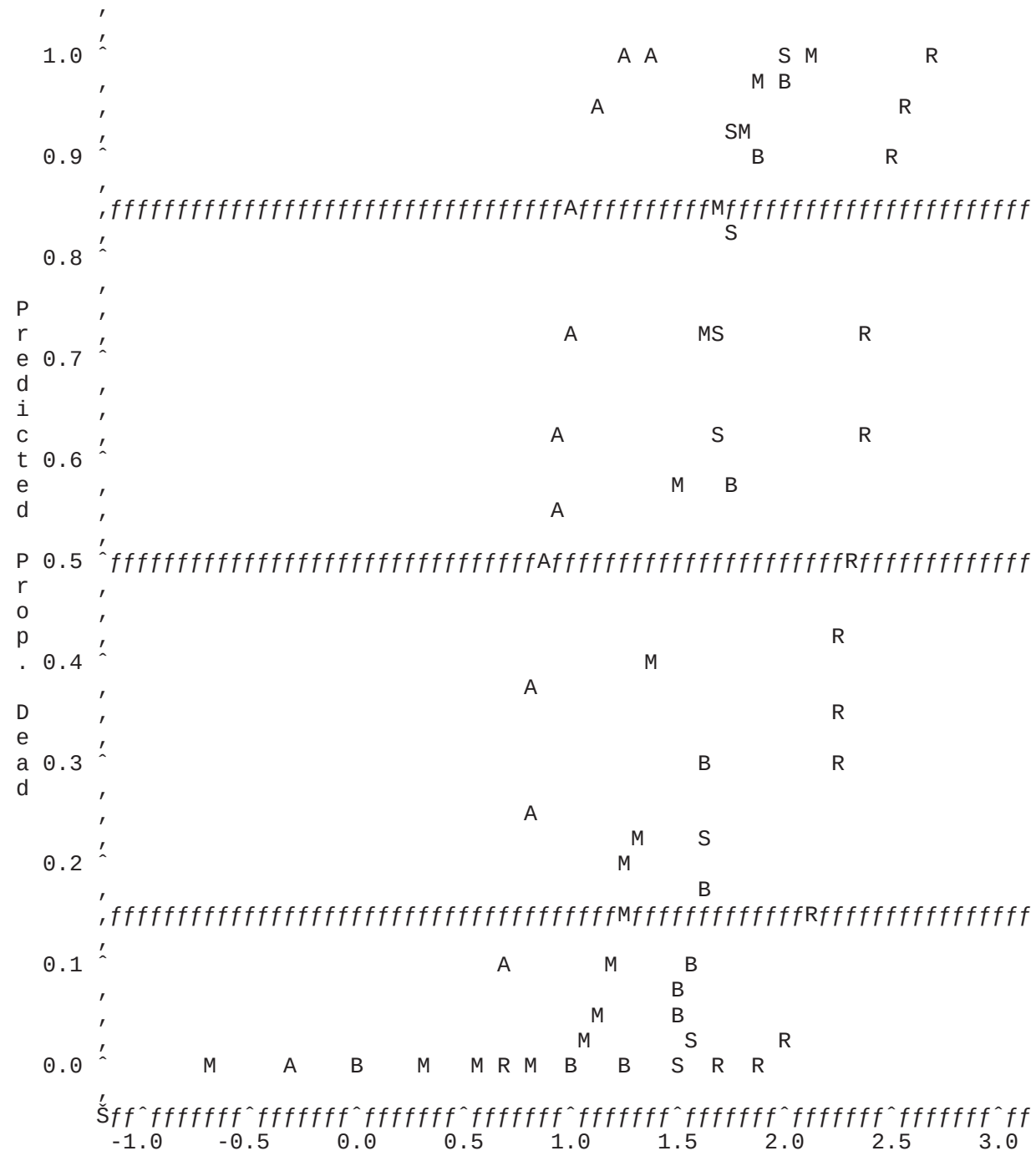
R-Square	C.V.	Root MSE	STUDRES Mean
0.030061	3595.104	0.911466	0.025353

Source	DF	Type I SS	Mean Square	F Value	Pr > F
STAGE	7	2.41936734	0.34562391	0.42	0.8903
LOGDOSE	1	0.10395317	0.10395317	0.13	0.7243

Source	DF	Type III SS	Mean Square	F Value	Pr > F
STAGE	7	2.51242328	0.35891761	0.43	0.8800
LOGDOSE	1	0.10395317	0.10395317	0.13	0.7243

SEPARATE-SLOPES DOSE-RESPONSE FITS
PROBABILITY PLOT OF PREDICTED PERCENT DEAD WITH 16%, 50%, 84% REFERENCE LINES

---------- Agent=GF ----------



SEPARATE-SLOPES DOSE-RESPONSE FITS
OUTPUT COEFFICIENTS AND COVARIANCE MATRIX FROM PROBIT REGRESSION

TRTGRP	SSE	RESIDSSQ	NTOT	NPTS	SLP	INT1	VARB1	COVB0B1	VARB0
Acontrol	28.5757	12.2426	28	18	6.9412	-1.1573	11.4245	-10.327	9.410
Bleach	20.2344	22.1207	28	21	7.4461	-7.8168	7.0766	-11.698	19.437
M291	22.3500	16.8114	35	27	4.4311	-1.4868	1.5797	-2.309	3.466
RSDL	25.2563	13.3881	32	24	6.9617	-11.0118	5.5056	-12.716	29.452
Soap	15.5481	11.5610	21	19	17.6020	-24.3612	69.6350	-117.579	198.667

SEPARATE-SLOPES DOSE-RESPONSE FITS
PERCENTILES WITH CONFIDENCE INTERVALS BASED ON FIELLER'S AND DELTA METHOD

---------- Agent=GF Treatment Group=Acontrol ----------

Agent	Perc-entile	Probit of Percentile	Log(Eff. Dose) for Percentile	Std. Error of Log(Eff. Dose)	Effective Dose for Percentile	Lower Confid-ence Bound	Upper Confid-ence Bound	LCBD	UCBD
GF	1	-2.32635	0.55192	0.17587	3.5639	0.0000	5.40	1.61142	7.8820
GF	10	-1.28155	0.70244	0.10571	5.0402	0.0003	6.55	3.12785	8.1216
GF	16	-0.99446	0.74380	0.08734	5.5438	0.0023	6.94	3.73782	8.2223
GF	30	-0.52440	0.81152	0.05977	6.4792	0.0696	7.76	4.94736	8.4855
GF	50	-0.00000	0.88707	0.04018	7.7103	2.5072	10.71	6.43158	9.2434
GF	70	0.52440	0.96262	0.04861	9.1754	7.6379	174.73	7.36781	11.4264
GF	84	0.99446	1.03034	0.07304	10.7237	8.7944	5031.47	7.71223	14.9110
GF	90	1.28155	1.07170	0.09066	11.7952	9.3609	40112.89	7.83431	17.7585
GF	99	2.32635	1.22223	0.15990	16.6811	11.4033	78942998.17	8.10633	34.3262

---------- Agent=GF Treatment Group=Bleach ----------

Agent	Perc-entile	Probit of Percentile	Log(Eff. Dose) for Percentile	Std. Error of Log(Eff. Dose)	Effective Dose for Percentile
GF	1	-2.32635	1.40886	0.097037	25.6367
GF	10	-1.28155	1.54918	0.056421	35.4142
GF	16	-0.99446	1.58773	0.048486	38.7020
GF	30	-0.52440	1.65086	0.042511	44.7571
GF	50	-0.00000	1.72129	0.049003	52.6366
GF	70	0.52440	1.79171	0.065279	61.9034
GF	84	0.99446	1.85484	0.083695	71.5885
GF	90	1.28155	1.89340	0.095817	78.2347

Lower Confidence Bound	Upper Confidence Bound	LCBD	UCBD
6.5884	33.817	16.5451	39.724
18.4051	43.012	27.4532	45.684
23.8628	47.001	31.0958	48.169
34.0442	58.278	36.9436	54.223
43.3760	86.428	42.1933	65.665
50.3158	140.786	46.1070	83.112
55.9980	223.775	49.0682	104.445
59.4832	298.462	50.7689	120.559

SEPARATE-SLOPES DOSE-RESPONSE FITS
PERCENTILES WITH CONFIDENCE INTERVALS BASED ON FIELLER'S AND DELTA METHOD

---------- Agent=GF Treatment Group=Bleach ----------
(continued)

Agent	Percentile	Probit of Percentile	Log(Eff. Dose) for Percentile	Std. Error of Log(Eff. Dose)	Effective Dose for Percentile
GF	99	2.32635	2.03371	0.14249	108.072

Lower Confidence Bound	Upper Confidence Bound	LCBD	UCBD
73.1261	862.617	56.8112	205.587

---------- Agent=GF Treatment Group=M291 ----------

Agent	Percentile	Probit of Percentile	Log(Eff. Dose) for Percentile	Std. Error of Log(Eff. Dose)	Effective Dose for Percentile
GF	1	-2.32635	0.93892	0.16324	8.6879
GF	10	-1.28155	1.17470	0.10626	14.9522
GF	16	-0.99446	1.23950	0.09294	17.3578
GF	30	-0.52440	1.34558	0.07585	22.1604
GF	50	-0.00000	1.46392	0.06835	29.1021
GF	70	0.52440	1.58227	0.07644	38.2182
GF	84	0.99446	1.68835	0.09386	48.7923
GF	90	1.28155	1.75314	0.10729	56.6425
GF	99	2.32635	1.98893	0.16446	97.4835

Lower Confidence Bound	Upper Confidence Bound	LCBD	UCBD
1.7980	14.305	4.1588	18.149
5.8196	21.276	9.2564	24.153
7.9352	24.031	11.4113	26.403
12.8081	30.194	15.7369	31.206
20.1292	42.281	21.3779	39.617
28.0615	66.748	27.0673	53.963
35.1841	107.962	31.9440	74.527
39.7139	147.307	34.9020	91.925
59.0100	477.275	46.4071	204.775

---------- Agent=GF Treatment Group=RSDL ----------

Agent	Perc-entile	Probit of Percentile	Log(Eff. Dose) for Percentile	Std. Error of Log(Eff. Dose)	Effective Dose for Percentile
GF	1	-2.32635	1.96581	0.12301	92.4290

Lower Confid-ence Bound	Upper Confid-ence Bound	LCBD	UCBD
19.1602	130.832	53.0538	161.027

SEPARATE-SLOPES DOSE-RESPONSE FITS
PERCENTILES WITH CONFIDENCE INTERVALS BASED ON FIELLER'S AND DELTA METHOD

---------- Agent=GF Treatment Group=RSDL ----------
(continued)

Agent	Perc-entile	Probit of Percentile	Log(Eff. Dose) for Percentile	Std. Error of Log(Eff. Dose)	Effective Dose for Percentile
GF	10	-1.28155	2.11589	0.07723	130.583
GF	16	-0.99446	2.15712	0.06589	143.590
GF	30	-0.52440	2.22464	0.05020	167.743
GF	50	-0.00000	2.29997	0.04134	199.513
GF	70	0.52440	2.37530	0.04678	237.299
GF	84	0.99446	2.44282	0.06093	277.215
GF	90	1.28155	2.48406	0.07179	304.828
GF	99	2.32635	2.63413	0.11687	430.658

Lower Confid-ence Bound	Upper Confid-ence Bound	LCBD	UCBD
51.821	164.88	92.154	185.036
67.659	176.88	106.652	193.320
102.836	202.06	133.738	210.394
152.848	251.57	165.552	240.439
196.717	361.73	192.140	293.073
227.169	543.84	210.568	364.956
244.394	708.07	220.473	421.459
309.121	1908.09	254.140	729.778

---------- Agent=GF Treatment Group=Soap ----------

Agent	Perc-entile	Probit of Percentile	Log(Eff. Dose) for Percentile	Std. Error of Log(Eff. Dose)	Effective Dose for Percentile
GF	1	-2.32635	1.53589	0.075266	34.3474
GF	10	-1.28155	1.59525	0.048837	39.3777
GF	16	-0.99446	1.61156	0.041966	40.8847
GF	30	-0.52440	1.63827	0.031588	43.4776
GF	50	-0.00000	1.66806	0.022901	46.5648
GF	70	0.52440	1.69785	0.021215	49.8712
GF	84	0.99446	1.72455	0.026880	53.0340
GF	90	1.28155	1.74086	0.032351	55.0636
GF	99	2.32635	1.80022	0.056880	63.1279

Lower Confidence Bound	Upper Confidence Bound	LCBD	UCBD
0.3368	41.23	24.4552	48.2410
2.3013	44.63	31.5886	49.0876
3.8936	45.72	33.8305	49.4099
9.1552	47.83	37.7011	50.1392
22.9851	52.02	41.9925	51.6349
42.6422	76.55	45.3177	54.8822
48.2657	166.41	46.9754	59.8741
50.0079	278.34	47.5836	63.7196
54.7634	1880.16	48.8355	81.6032

ICD CANDIDATE TREATMENTS FOR GF - COMPARISONS AMONG LD50s
ESTIMATED BY SRT SLOPES PROBIT ANALYSES MODEL 95% C.I.
PERCENTILE ESTIMATES OF DOSES PRODUCING SPECIFIED RESPONSE
RATES SHOWN WITH DELTA-TYPE CONFIDENCE INTERVALS

---------- Agent=GF Percentile=0.5 ----------

Treatment Group	Log(Leth Dose) for Percentile	Standard Error for Log(L.D.)	Log (Lower Conf. Bnd)	Log (Upper Conf. Bnd)	Leth Dose for Percentile	Lower Confid-ence Bound	Upper Confid-ence Bound
Acontrol	0.88707	0.040182	0.80832	0.96583	7.710	6.432	9.243
Bleach	1.72129	0.049003	1.62524	1.81733	52.637	42.193	65.665
M291	1.46392	0.068346	1.32997	1.59788	29.102	21.378	39.617
RSDL	2.29997	0.041344	2.21894	2.38100	199.513	165.552	240.439
Soap	1.66806	0.022901	1.62317	1.71294	46.565	41.993	51.635

ICD CANDIDATE TREATMENTS FOR GF - COMPARISONS AMONG LD50s
ESTIMATED BY SRT SLOPES PROBIT ANALYSES MODEL 95% C.I.
PROTECTIVE RATIOS AND CONFIDENCE BOUNDS FOR SPECIFIED PERCENTILES

---------- Agent=GF Percentile=0.5 ----------

1st Group (Denominator)	2nd Group (Numerator)	Log(L.D.), 1st Group	Log(L.D.), 2nd Group	Del(LogLD) 2nd - 1st	Std Err, Delta	Protective Ratio	Lower Confid-ence Bound	Upper Confid-ence Bound
Acontrol	Bleach	0.88707	1.72129	0.83421	0.063371	6.8268	5.1287	9.0870
Acontrol	M291	0.88707	1.46392	0.57685	0.079283	3.7744	2.6391	5.3981
Acontrol	RSDL	0.88707	2.29997	1.41290	0.057653	25.8760	19.9478	33.5658
Acontrol	Soap	0.88707	1.66806	0.78098	0.046250	6.0393	4.9016	7.4410
Bleach	M291	1.72129	1.46392	-0.25736	0.084098	0.5529	0.3783	0.8081
Bleach	RSDL	1.72129	2.29997	0.57868	0.064114	3.7904	2.8380	5.0623
Bleach	Soap	1.72129	1.66806	-0.05323	0.054090	0.8846	0.6930	1.1292
M291	RSDL	1.46392	2.29997	0.83605	0.079878	6.8556	4.7806	9.8312
M291	Soap	1.46392	1.66806	0.20413	0.072081	1.6001	1.1557	2.2152
RSDL	Soap	2.29997	1.66806	-0.63191	0.047263	0.2334	0.1886	0.2889

ICD CANDIDATE TREATMENTS FOR GF - COMPARISONS AMONG LD50s
ESTIMATED BY SRT SLOPES PROBIT ANALYSES MODEL AT 99.5% CI
PERCENTILE ESTIMATES OF DOSES PRODUCING SPECIFIED RESPONSE
RATES SHOWN WITH DELTA-TYPE CONFIDENCE INTERVALS

---------- Agent=GF Percentile=0.5 ----------

Treatment Group	Log(Leth Dose) for Percentile	Standard Error for Log(L.D.)	Log (Lower Conf. Bnd)	Log (Upper Conf. Bnd)	Leth Dose for Percentile	Lower Confid-ence Bound	Upper Confid-ence Bound
Acontrol	0.88707	0.040182	0.77404	1.00011	7.710	5.944	10.002
Bleach	1.72129	0.049003	1.58344	1.85913	52.637	38.322	72.299
M291	1.46392	0.068346	1.27167	1.65618	29.102	18.692	45.309
RSDL	2.29997	0.041344	2.18367	2.41627	199.513	152.640	260.778
Soap	1.66806	0.022901	1.60364	1.73248	46.565	40.146	54.010

ICD CANDIDATE TREATMENTS FOR GF - COMPARISONS AMONG LD50s
ESTIMATED BY SRT SLOPES PROBIT ANALYSES MODEL AT 99.5% CI
PROTECTIVE RATIOS AND CONFIDENCE BOUNDS FOR SPECIFIED PERCENTILES

---------- Agent=GF Percentile=0.5 ----------

1st Group (Denominator)	2nd Group (Numerator)	Log(L.D.), 1st Group	Log(L.D.), 2nd Group	Del(LogLD) 2nd - 1st	Std Err, Delta	Protective Ratio	Lower Confid-ence Bound	Upper Confid-ence Bound
Acontrol	Bleach	0.88707	1.72129	0.83421	0.063371	6.8268	4.5285	10.2914
Acontrol	M291	0.88707	1.46392	0.57685	0.079283	3.7744	2.2585	6.3077
Acontrol	RSDL	0.88707	2.29997	1.41290	0.057653	25.8760	17.8122	37.5903
Acontrol	Soap	0.88707	1.66806	0.78098	0.046250	6.0393	4.4759	8.1486
Bleach	M291	1.72129	1.46392	-0.25736	0.084098	0.5529	0.3207	0.9532
Bleach	RSDL	1.72129	2.29997	0.57868	0.064114	3.7904	2.5022	5.7416
Bleach	Soap	1.72129	1.66806	-0.05323	0.054090	0.8846	0.6232	1.2558
M291	RSDL	1.46392	2.29997	0.83605	0.079878	6.8556	4.0865	11.5012
M291	Soap	1.46392	1.66806	0.20413	0.072081	1.6001	1.0032	2.5521
RSDL	Soap	2.29997	1.66806	-0.63191	0.047263	0.2334	0.1718	0.317011:37

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SAS ANALYSIS USING PROBSEPX PROGRAM FOR DELAYED DECON GF LT_{50}
Final Report After complete QC Audit of Data

File: GF All Delayed Decons SAS output 150708.doc

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                                    LT50 GF ALL DELAYED DECONS                          1
                                                14:33 Wednesday, July 8, 2015
                     SEPARATE-SLOPES DOSE-RESPONSE FITS
        PROBIT FITS TO DATA USING ALL EXPERIMENTAL STAGES, LOG10(DOSE)

----------------------- AGENT=GF TRTGRP=Bleach delay -----------------------

     Non-Linear Least Squares Grid Search     Dependent Variable NDEAD
                         B1             B01       Weighted loss
                         0.216162       6.410109      24.180447

                   Non-Linear Least Squares Iterative Phase
               Dependent Variable NDEAD   Method: Gauss-Newton
              Iter       B1             B01       Weighted loss
                 0       0.216162       6.410109      24.180447
                 1       1.218039       5.841321      19.797270
                 2       1.465384       6.101514      18.482444
                 3       1.806350       6.207884      18.184481
                 4       1.987948       6.265973      18.136399
                 5       2.033380       6.282439      18.133803
                 6       2.039895       6.284913      18.133744
                 7       2.040674       6.285208      18.133743
                 8       2.040763       6.285243      18.133743
NOTE: Convergence criterion met.


   Non-Linear Least Squares Summary Statistics     Dependent Variable NDEAD

       Source                DF    Weighted SS     Weighted MS

       Regression             2   643488.91059    321744.45530
       Residual              21       12.15629         0.57887
       Uncorrected Total     23   643501.06689

       (Corrected Total)     22    68161.04973
       Sum of Loss                    18.13374

       Parameter     Estimate    Asymptotic             Asymptotic 95 %
                                 Std. Error         Confidence Interval
                                                    Lower         Upper
       B1         2.040763294  1.1112765678 -0.2702448119  4.3517713998
       B01        6.285242647  0.4085616214  5.4355988712  7.1348864224


                       Asymptotic Correlation Matrix

                  Corr                B1               B01
                  ffffffffffffffffffffffffffffffffffffffff
                  B1                   1      0.5607605177
                  B01       0.5607605177                 1
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SEPARATE-SLOPES DOSE-RESPONSE FITS
PROBIT FITS TO DATA USING ALL EXPERIMENTAL STAGES, LOG10(DOSE)

---------- AGENT=GF TRTGRP=RSDL delay ----------

Non-Linear Least Squares Grid Search Dependent Variable NDEAD

B1	B01	Weighted loss
3.027517	0.415731	31.633458

Non-Linear Least Squares Iterative Phase
Dependent Variable NDEAD Method: Gauss-Newton

Iter	B1	B01	Weighted loss
0	3.027517	0.415731	31.633458
1	5.410711	-3.263579	26.007860
2	7.169998	-6.002090	24.501833
3	8.043931	-7.372155	24.287980
4	8.254387	-7.704407	24.278448
5	8.276777	-7.740051	24.278339
6	8.278524	-7.742837	24.278338
7	8.278656	-7.743047	24.278338

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable NDEAD

Source	DF	Weighted SS	Weighted MS
Regression	2	3525.0306263	1762.5153131
Residual	13	8.1344977	0.6257306
Uncorrected Total	15	3533.1651240	
(Corrected Total)	14	3477.7408381	
Sum of Loss		24.2783378	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
B1	8.278655784	2.4119390935	3.067981285	13.489330283
B01	-7.743046969	3.7427194407	-15.828695898	0.342601959

Asymptotic Correlation Matrix

Corr	B1	B01
B1	1	-0.996966898
B01	-0.996966898	1

SEPARATE-SLOPES DOSE-RESPONSE FITS
PROBIT FITS TO DATA USING ALL EXPERIMENTAL STAGES, LOG10(DOSE)

---------- AGENT=GF TRTGRP=Soap delay ----------

Non-Linear Least Squares Grid Search Dependent Variable NDEAD

B1	B01	Weighted loss
0.002333	6.499215	14.279688

Non-Linear Least Squares Iterative Phase
Dependent Variable NDEAD Method: Gauss-Newton

Iter	B1	B01	Weighted loss
0	0.002333	6.499215	14.279688
1	0.077292	6.403630	14.233681
2	0.073617	6.410061	14.233441
3	0.073536	6.410119	14.233441

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable NDEAD

Source	DF	Weighted SS	Weighted MS
Regression	2	341.96888759	170.98444379
Residual	15	8.44876858	0.56325124
Uncorrected Total	17	350.41765617	
(Corrected Total)	16	64.57320072	
Sum of Loss		14.23344135	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
B1	0.073535733	0.45964104304	-0.9061622343	1.0532337012
B01	6.410118857	0.41957473570	5.5158198773	7.3044178370

Asymptotic Correlation Matrix

Corr	B1	B01
B1	1	-0.514145838
B01	-0.514145838	1

SEPARATE-SLOPES DOSE-RESPONSE FITS
OUTPUT DATA FROM PROC NLIN -- LISTING OF PREDICTED VALUES ANDRESIDUALS

---------- Agent=GF Treatment Group=Bleach delay ----------

OBS	STAGE	Agent Dose (mg/kg)	Log10 Dose (mg/kg)	No. Animals	Observed N Dead	Prop. Dead	Probit of Percentile	LCL
1	5	0.25	-.602	3	3	1.000	1.64485	0.18876
2	3	0.50	-.301	1	1	1.000	1.64485	0.51416
3	4	0.50	-.301	5	1	0.200	-0.84162	2.57082
4	3	0.67	-.174	1	1	1.000	1.64485	0.64082
5	3	1.00	0.000	1	1	1.000	1.64485	0.75225
6	5	1.00	0.000	3	3	1.000	1.64485	2.25676
7	6	1.00	0.000	2	2	1.000	1.64485	1.50451
8	3	1.67	0.223	1	1	1.000	1.64485	0.85230
9	2	2.00	0.301	1	1	1.000	1.64485	0.88149
10	5	2.00	0.301	3	3	1.000	1.64485	2.64448
11	3	2.67	0.427	1	1	1.000	1.64485	0.92105
12	3	4.17	0.620	1	1	1.000	1.64485	0.96325
13	1	5.00	0.699	1	1	1.000	1.64485	0.97437
14	2	5.00	0.699	2	2	1.000	1.64485	1.94874
15	3	6.67	0.824	1	1	1.000	1.64485	0.98636
16	2	10.00	1.000	2	2	1.000	1.64485	1.99003
17	3	10.50	1.021	1	1	1.000	1.64485	0.99563
18	1	15.00	1.176	2	2	1.000	1.64485	1.99684
19	2	15.00	1.176	2	2	1.000	1.64485	1.99684
20	1	30.00	1.477	2	2	1.000	1.64485	1.99968
21	2	30.00	1.477	1	1	1.000	1.64485	0.99984
22	1	45.00	1.653	2	2	1.000	1.64485	1.99993

OBS	UCL	Predicted No. Dead	Studentized Residuals	Predicted Prop. Dead	Probit Pred. Pct Dead
1	2.94661	1.56768	2.57741	0.52256	0.05658
2	0.98356	0.74886	0.59975	0.74886	0.67091
3	4.91779	3.74431	-3.47984	0.74886	0.67091
4	1.00696	0.82389	0.47518	0.82389	0.93030
5	1.04904	0.90065	0.34199	0.90065	1.28524
6	3.14711	2.70194	0.63170	0.90065	1.28524
7	2.09808	1.80129	0.49894	0.90065	1.28524
8	1.06579	0.95905	0.21391	0.95905	1.64485
9	1.06102	0.97126	0.17804	0.97126	1.64485
10	3.18306	2.91377	0.33316	0.97126	1.64485
11	1.04783	0.98444	0.12965	0.98444	1.64485
12	1.02600	0.99463	0.07505	0.99463	1.64485
13	1.01893	0.99665	0.05888	0.99665	1.64485
14	2.03787	1.99331	0.08486	0.99665	1.64485
15	1.01063	0.99850	0.03911	0.99850	1.64485
16	2.00820	1.99912	0.02984	0.99956	1.64485
17	1.00362	0.99962	0.01926	0.99962	1.64485
18	2.00270	1.99977	0.01485	0.99989	1.64485
19	2.00270	1.99977	0.01485	0.99989	1.64485
20	2.00029	1.99998	0.00329	0.99999	1.64485
21	1.00014	0.99999	0.00199	0.99999	1.64485
22	2.00006	2.00000	0.00088	1.00000	1.64485

SEPARATE-SLOPES DOSE-RESPONSE FITS
OUTPUT DATA FROM PROC NLIN -- LISTING OF PREDICTED VALUES ANDRESIDUALS

---------- Agent=GF Treatment Group=Bleach delay ---------- (continued)

OBS	STAGE	Agent Dose (mg/kg)	Log10 Dose (mg/kg)	No. Animals	Observed N Dead	Prop. Dead	Probit of Percentile	LCL
23	1	60.00	1.778	1	1	1.000	1.64485	0.99999

OBS	UCL	Predicted No. Dead	Studentized Residuals	Predicted Prop. Dead	Probit Pred. Pct Dead
23	1.00001	1.00000	0.00014	1.00000	1.64485

---------- Agent=GF Treatment Group=RSDL delay ----------

OBS	STAGE	Agent Dose (mg/kg)	Log10 Dose (mg/kg)	No. Animals	Observed N Dead	Prop. Dead	Probit of Percentile	LCL
24	1	5	0.699	1	0	0.000	-1.64485	-0.00000
25	3	5	0.699	1	0	0.000	-1.64485	-0.00000
26	1	15	1.176	1	0	0.000	-1.64485	-0.00751
27	2	15	1.176	1	0	0.000	-1.64485	-0.00751
28	3	15	1.176	2	0	0.000	-1.64485	-0.01502
29	3	20	1.301	4	0	0.000	-1.64485	-0.22867
30	3	25	1.398	4	0	0.000	-1.64485	-0.32060
31	1	30	1.477	1	1	1.000	1.64485	0.04936
32	2	30	1.477	5	2	0.400	-0.25335	0.24681
33	3	40	1.602	4	3	0.750	0.67449	1.82976
34	1	45	1.653	1	0	0.000	-1.64485	0.61305
35	2	45	1.653	6	5	0.833	0.96742	3.67827
36	1	60	1.778	1	1	1.000	1.64485	0.89933
37	2	60	1.778	4	4	1.000	1.64485	3.59732

OBS	UCL	Predicted No. Dead	Studentized Residuals	Predicted Prop. Dead	Probit Pred. Pct Dead
24	0.00000	0.00000	-0.00000	0.00000	-1.64485
25	0.00000	0.00000	-0.00000	0.00000	-1.64485
26	0.01015	0.00132	-0.03646	0.00132	-1.64485
27	0.01015	0.00132	-0.03646	0.00132	-1.64485
28	0.02030	0.00264	-0.05200	0.00132	-1.64485
29	0.42299	0.09716	-0.36193	0.02429	-1.64485
30	1.28864	0.48402	-0.90389	0.12100	-1.16998
31	0.55756	0.30346	1.56714	0.30346	-0.51447
32	2.78781	1.51731	0.57244	0.30346	-0.51447
33	3.75758	2.79367	0.25722	0.69842	0.51986
34	1.04144	0.82724	-2.26756	0.82724	0.94333
35	6.24866	4.96346	0.05149	0.82724	0.94333
36	1.05270	0.97602	0.16112	0.97602	1.64485
37	4.21081	3.90406	0.35390	0.97602	1.64485

SEPARATE-SLOPES DOSE-RESPONSE FITS
OUTPUT DATA FROM PROC NLIN -- LISTING OF PREDICTED VALUES ANDRESIDUALS

---------- Agent=GF Treatment Group=RSDL delay ----------
(continued)

OBS	STAGE	Agent Dose (mg/kg)	Log10 Dose (mg/kg)	No. Animals	Observed N Dead	Prop. Dead	Probit of Percentile	LCL
38	1	90	1.954	1	1	1.000	1.64485	0.99729

OBS	UCL	Predicted No. Dead	Studentized Residuals	Predicted Prop. Dead	Probit Pred. Pct Dead
38	1.00212	0.99970	0.016952	0.99970	1.64485

---------- Agent=GF Treatment Group=Soap delay ----------

OBS	STAGE	Agent Dose (mg/kg)	Log10 Dose (mg/kg)	No. Animals	Observed N Dead	Prop. Dead	Probit of Percentile	LCL
39	3	0.50	-.301	1	1	1.000	1.64485	0.75360
40	4	0.50	-.301	5	4	0.800	0.84162	3.76802
41	5	0.50	-.301	4	4	1.000	1.64485	3.01442
42	3	0.67	-.174	1	1	1.000	1.64485	0.76966
43	3	1.00	0.000	1	1	1.000	1.64485	0.78875
44	3	1.67	0.223	1	1	1.000	1.64485	0.80716
45	3	2.67	0.427	1	1	1.000	1.64485	0.81685
46	3	4.17	0.620	1	1	1.000	1.64485	0.81926
47	1	5.00	0.699	1	1	1.000	1.64485	0.81847
48	3	6.67	0.824	1	1	1.000	1.64485	0.81541
49	3	10.50	1.021	1	1	1.000	1.64485	0.80701
50	1	15.00	1.176	2	1	0.500	-0.00000	1.59649
51	1	30.00	1.477	2	2	1.000	1.64485	1.55637
52	2	30.00	1.477	1	1	1.000	1.64485	0.77819

OBS	UCL	Predicted No. Dead	Studentized Residuals	Predicted Prop. Dead	Probit Pred. Pct Dead
39	1.08125	0.91743	0.31241	0.91743	1.38798
40	5.40627	4.58714	-1.22140	0.91743	1.38798
41	4.32501	3.66972	0.72332	0.91743	1.38798
42	1.06802	0.91884	0.30744	0.91884	1.39733
43	1.05275	0.92075	0.30139	0.92075	1.41012
44	1.03911	0.92314	0.29475	0.92314	1.42650
45	1.03370	0.92528	0.28963	0.92528	1.44148
46	1.03527	0.92727	0.28554	0.92727	1.45572
47	1.03765	0.92806	0.28407	0.92806	1.46152
48	1.04322	0.92932	0.28196	0.92932	1.47072
49	1.05550	0.93126	0.27918	0.93126	1.48521
50	2.13451	1.86550	-2.61515	0.93275	1.49660
51	2.18597	1.87117	0.41001	0.93559	1.51874
52	1.09299	0.93559	0.27511	0.93559	1.51874

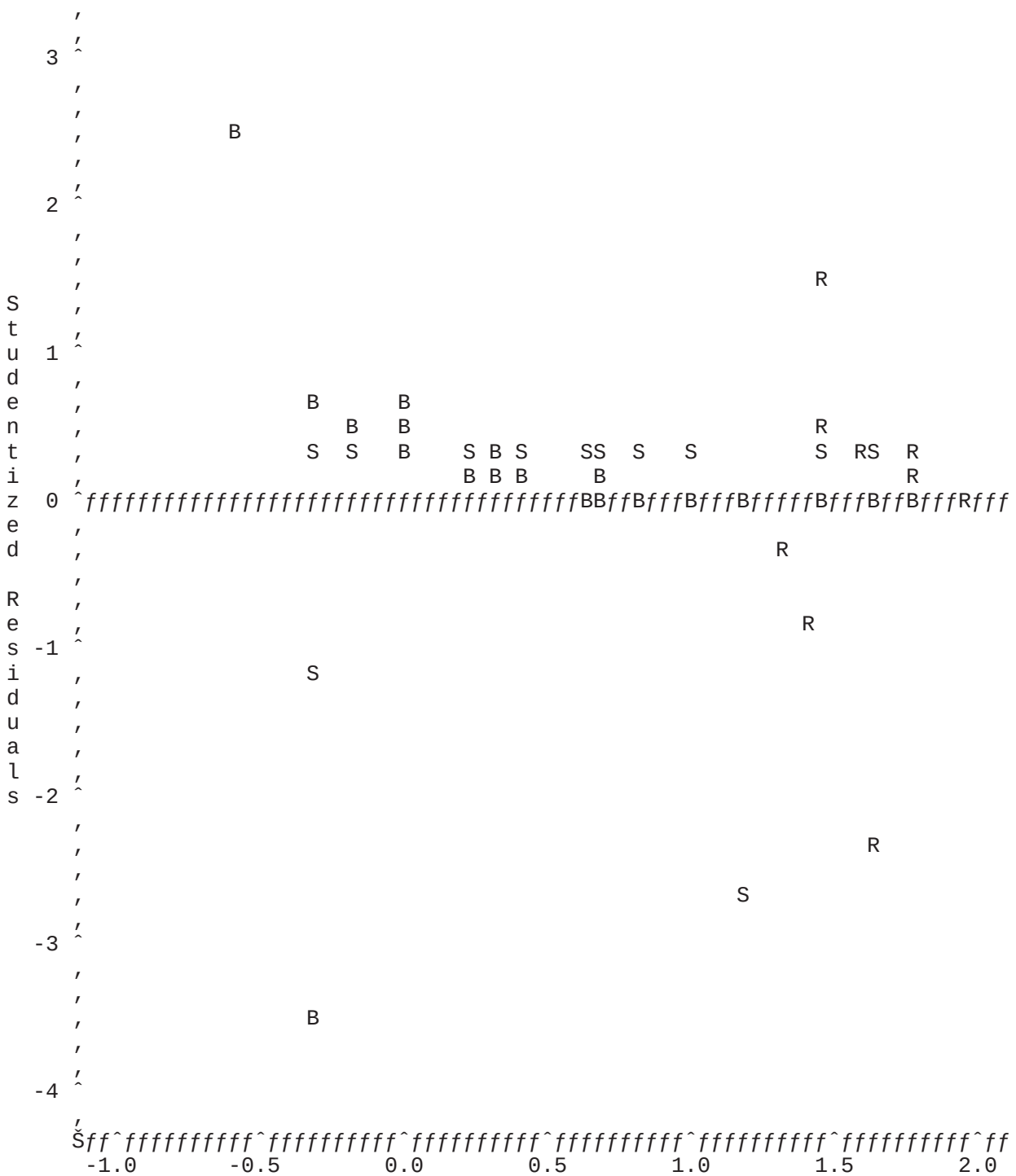
SEPARATE-SLOPES DOSE-RESPONSE FITS
OUTPUT DATA FROM PROC NLIN -- LISTING OF PREDICTED VALUES ANDRESIDUALS

---------- Agent=GF Treatment Group=Soap delay ----------
(continued)

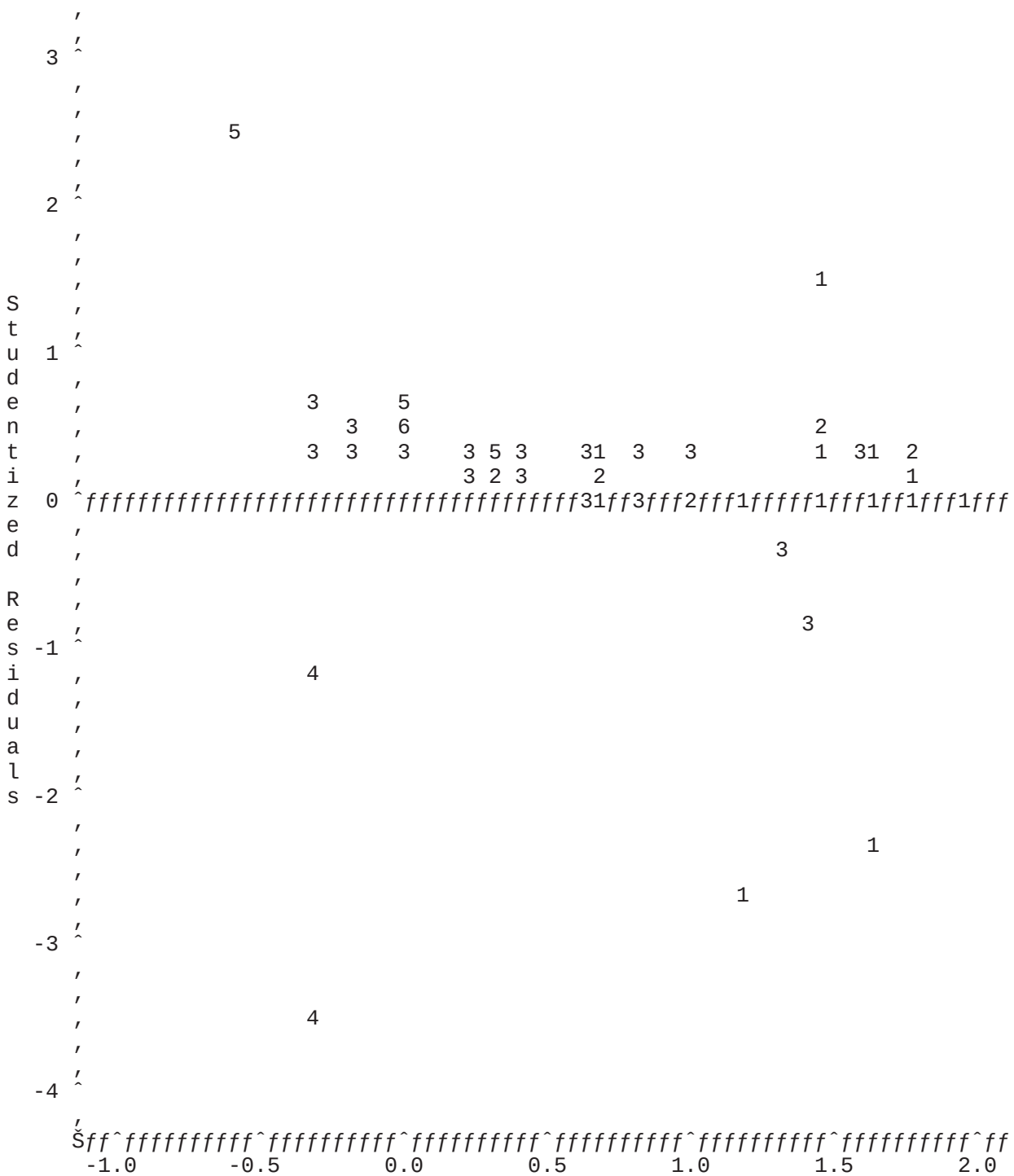
OBS	STAGE	Agent Dose (mg/kg)	Log10 Dose (mg/kg)	No. Animals	Observed N Dead	Prop. Dead	Probit of Percentile	LCL
53	2	40	1.602	1	1	1.000	1.64485	0.76926
54	1	45	1.653	2	2	1.000	1.64485	1.53113
55	1	60	1.778	1	1	1.000	1.64485	0.75649

OBS	UCL	Predicted No. Dead	Studentized Residuals	Predicted Prop. Dead	Probit Pred. Pct Dead
53	1.10421	0.93673	0.27455	0.93673	1.52793
54	2.21767	1.87440	0.41457	0.93720	1.53169
55	1.12017	0.93833	0.27417	0.93833	1.54088

SEPARATE-SLOPES DOSE-RESPONSE FITS
OUTPUT DATA FROM PROC NLIN -- LISTING OF PREDICTED VALUES ANDRESIDUALS
RESIDUALS VS. LOGDOSE - PLOTTING SYMBOL IS TREATMENT GROUP



SEPARATE-SLOPES DOSE-RESPONSE FITS
OUTPUT DATA FROM PROC NLIN -- LISTING OF PREDICTED VALUES ANDRESIDUALS
RESIDUALS VS. LOGDOSE - PLOTTING SYMBOL IS EXPERIMENTAL STAGE



SEPARATE-SLOPES DOSE-RESPONSE FITS
OUTPUT DATA FROM PROC NLIN -- LISTING OF PREDICTED VALUES ANDRESIDUALS
DESCRIPTIVE STATISTICS OF RESIDUALS BY STAGE

Analysis Variable : STUDRES Studentized Residuals

---------- Agent=GF Experimental Stage=1 ----------

Mean	N	Std Dev	Std Error	Minimum	Maximum
-0.1070691	16	0.9926557	0.2481639	-2.6151497	1.5671395

---------- Agent=GF Experimental Stage=2 ----------

Mean	N	Std Dev	Std Error	Minimum	Maximum
0.1636916	11	0.1879477	0.0566684	-0.0364620	0.5724361

---------- Agent=GF Experimental Stage=3 ----------

Mean	N	Std Dev	Std Error	Minimum	Maximum
0.1516956	21	0.3161389	0.0689872	-0.9038861	0.5997508

---------- Agent=GF Experimental Stage=4 ----------

Mean	N	Std Dev	Std Error	Minimum	Maximum
-2.3506188	2	1.5969627	1.1292232	-3.4798420	-1.2213957

---------- Agent=GF Experimental Stage=5 ----------

Mean	N	Std Dev	Std Error	Minimum	Maximum
1.0663985	4	1.0210245	0.5105122	0.3331563	2.5774134

---------- Agent=GF Experimental Stage=6 ----------

Mean	N	Std Dev	Std Error	Minimum	Maximum
0.4989427	1	.	.	0.4989427	0.4989427

---------- Agent=GF ----------

General Linear Models Procedure
Class Level Information

Class	Levels	Values
STAGE	6	1 2 3 4 5 6

Number of observations in by group = 55

---------- Agent=GF ----------

General Linear Models Procedure

Dependent Variable: STUDRES Studentized Residuals

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	17.37791429	2.89631905	6.31	0.0001
Error	48	22.04004740	0.45916765		
Corrected Total	54	39.41796168			

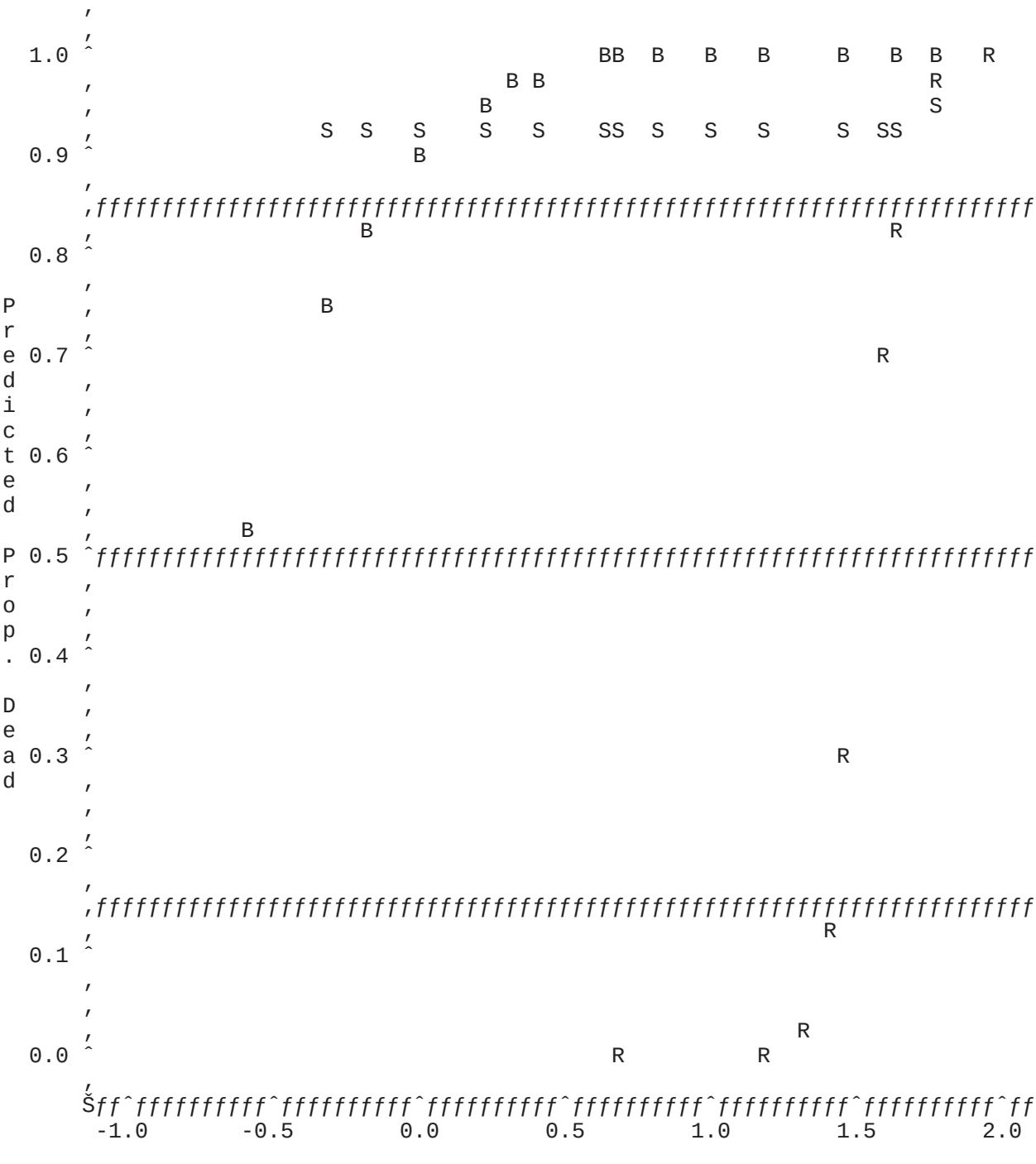
R-Square	C.V.	Root MSE	STUDRES Mean
0.440863	1117.041	0.677619	0.060662

Source	DF	Type I SS	Mean Square	F Value	Pr > F
STAGE	5	16.60759892	3.32151978	7.23	0.0001
LOGDOSE	1	0.77031537	0.77031537	1.68	0.2014

Source	DF	Type III SS	Mean Square	F Value	Pr > F
STAGE	5	16.94978661	3.38995732	7.38	0.0001
LOGDOSE	1	0.77031537	0.77031537	1.68	0.2014

SEPARATE-SLOPES DOSE-RESPONSE FITS
PROBABILITY PLOT OF PREDICTED PERCENT DEAD WITH 16%, 50%, 84% REFERENCE LINES

---------- Agent=GF ----------



SEPARATE-SLOPES DOSE-RESPONSE FITS
OUTPUT COEFFICIENTS AND COVARIANCE MATRIX FROM PROBIT REGRESSION

TRTGRP	SSE	RESIDSSQ	NOTS	NPTS	SLP	INTB1	VARB1	COVB01	VARB0
Bleach delay	18.1337	12.1563	40	23	2.04076	6.28524	1.23494	0.25460	0.1669
RSDL delay	24.2783	8.1345	37	15	8.27866	-7.74305	5.81745	-8.99983	14.0079
Soap delay	14.2334	8.4488	27	17	0.07354	6.41012	0.21127	-0.09915	0.1760

SEPARATE-SLOPES DOSE-RESPONSE FITS
PERCENTILES WITH CONFIDENCE INTERVALS BASED ON FIELLER'S AND DELTA METHOD

Agent=GF Treatment Group=Bleach delay

Agent	Perc-entile	Probit of Percentile	Log(Eff. Dose) for Percentile	Std. Error of Log(Eff. Dose)	Effective Dose for Percentile	Lower Confid-ence Bound	Upper Confid-ence Bound	LCBD	UCBD
GF	1	-2.32635	-1.76973	0.86741	0.01699	9.900424107E22	0.12	0.00034	0.8519
GF	10	-1.28155	-1.25776	0.59615	0.05524	2.35896885E15	0.21	0.00375	0.8141
GF	16	-0.99446	-1.11708	0.52300	0.07637	18835636401063	0.26	0.00721	0.8091
GF	30	-0.52440	-0.88675	0.40599	0.12979	6818608592.97	0.34	0.02077	0.8109
GF	50	-0.00000	-0.62979	0.28406	0.23454	932526.12	0.51	0.06508	0.8452
GF	70	0.52440	-0.37282	0.18898	0.42382	84.65	1.14	0.18063	0.9944
GF	84	0.99446	-0.14249	0.16935	0.72030	-999999.00	999999.00	0.33542	1.5468
GF	90	1.28155	-0.00181	0.19965	0.99584	0.43	0.00	0.40446	2.4519
GF	99	2.32635	0.51015	0.42382	3.23709	1.18	0.00	0.47803	21.9205

---------- Agent=GF Treatment Group=RSDL delay ----------

Agent	Perc-entile	Probit of Percentile	Log(Eff. Dose) for Percentile	Std. Error of Log(Eff. Dose)	Effective Dose for Percentile	Lower Confid-ence Bound	Upper Confid-ence Bound	LCBD	UCBD
GF	1	-2.32635	1.25826	0.091195	18.1242	7.2413	23.8365	12.0093	27.3529
GF	10	-1.28155	1.38446	0.059004	24.2361	13.9460	29.3190	18.5701	31.6309
GF	16	-0.99446	1.41914	0.051249	26.2508	16.6012	31.2159	20.8302	33.0819
GF	30	-0.52440	1.47592	0.040833	29.9172	21.7834	35.0667	24.8823	35.9711
GF	50	-0.00000	1.53927	0.035258	34.6151	28.2644	41.6646	29.5230	40.5855
GF	70	0.52440	1.60261	0.038731	40.0506	34.1346	53.1860	33.6276	47.7003
GF	84	0.99446	1.65939	0.048056	45.6445	38.6178	69.2963	36.7451	56.6993
GF	90	1.28155	1.69407	0.055433	49.4387	41.2050	82.3123	38.4962	63.4916

SEPARATE-SLOPES DOSE-RESPONSE FITS
PERCENTILES WITH CONFIDENCE INTERVALS BASED ON FIELLER'S AND DELTA METHOD

---------- Agent=GF Treatment Group=RSDL delay ----------
(continued)

Agent	Perc-entile	Probit of Percentile	Log(Eff. Dose) for Percentile	Std. Error of Log(Eff. Dose)	Effective Dose for Percentile
GF	99	2.32635	1.82027	0.087033	66.1105

Lower Confid-ence Bound	Upper Confid-ence Bound	LCBD	UCBD
50.8380	158.038	44.6360	97.9165

---------- Agent=GF Treatment Group=Soap delay ----------

Agent	Perc-entile	Probit of Percentile	Log(Eff. Dose) for Percentile	Std. Error of Log(Eff. Dose)	Effective Dose for Percentile
GF	1	-2.32635	-50.8116	320.573	-999999.00
GF	10	-1.28155	-36.6036	231.779	-999999.00
GF	16	-0.99446	-32.6994	207.382	-999999.00
GF	30	-0.52440	-26.3072	167.440	0.00
GF	50	-0.00000	-19.1760	122.892	0.00
GF	70	0.52440	-12.0447	78.373	0.00
GF	84	0.99446	-5.6525	38.577	0.00
GF	90	1.28155	-1.7484	14.700	0.02
GF	99	2.32635	12.4596	75.106	2.88166E12

Lower Confid-ence Bound	Upper Confid-ence Bound	LCBD	UCBD
89804.89	0.00	0	.
4584.97	0.01	0	.
2003.10	0.01	0	.
506.07	0.04	0	7.5158E301
102.65	0.16	9.0309E-261	4.9242E221
16.48	0.78	2.208E-166	3.6859E141
-999999.00	999999.00	5.46024E-82	9.07365E69
-999999.00	999999.00	2.74731E-31	1.15975E27
9.63	0.62	1.7857E-135	4.6503E

APENDIX C: EPA LIMIT TEST OUTPUT FILES

EPA LIMIT TEST USING AOT425 STATPGM (version: 1.0) ANALYSIS FOR SERPACWA GF LD_{50}
Final Report After complete QC Audit of Data

File: GF SERPACWA Statistical Analysis Output 061018 Final.doc

```
AOT425statpgm (Version: 1.0) Test Results and Recommendations
Acute Oral Toxicity (OECD Test Guideline 425) Statistical Program

Date/Time: Tuesday, August 29, 2006, 10:43:35 AM
Data file name: braue2.dat
Last modified: 8/29/2006 10:43:32 AM

Test/Substance: Enter test description.
Test type: Limit Test
Limit dose (mg/kg): 2000
Assumed LD50  (mg/kg): Default
Assumed sigma (mg/kg): 0.5

DATA:
```

Test Seq.	Animal ID	Dose (mg/kg)	Short-term Result	Long-term Result
1	1	250	O	O
2	2	250	O	O
3	3	250	X	X
4	4	250	O	O
5	5	250	O	O

(X = Died, O = Survived)

```
Dose Recommendation: The limit test is complete.

WARNING:
Please review the data for accuracy.
The limit test data includes doses well below the limit dose.

SUMMARY OF LONG-TERM RESULTS:
```

Dose	O	X	Total
250	4	1	5
All Doses	4	1	5

```
Statistical Estimates:

The LD50 is greater than 250 mg/kg.
```

AOT425statpgm (Version: 1.0) Test Results and Recommendations
Acute Oral Toxicity (OECD Test Guideline 425) Statistical Program

Date/Time: Tuesday, August 29, 2006, 10:41:47 AM
Data file name: braue.dat
Last modified: 8/29/2006 10:41:46 AM

Test/Substance: Enter test description.
Test type: Limit Test
Limit dose (mg/kg): 2000
Assumed LD50 (mg/kg): Default
Assumed sigma (mg/kg): 0.5

DATA:

Test Seq.	Animal ID	Dose (mg/kg)	Short-term Result	Long-term Result
1	1	250	O	O
2	2	250	O	O
3	3	250	O	O
4	4	250	O	O
5	5	250	O	O

(X = Died, O = Survived)

Dose Recommendation: The limit test is complete.

WARNING:
Please review the data for accuracy.
The limit test data includes doses well below the limit dose.

SUMMARY OF LONG-TERM RESULTS:

Dose	O	X	Total
250	5	0	5
All Doses	5	0	5

Statistical Estimates:

The LD50 is greater than 250 mg/kg.